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OBSERVATIONS UPON THE OCCURRENCE OF AN ANTIDIURETIC SUBSTANCE IN THE URINE OF PATIENTS WITH PRE-ECLAMPSIA AND ECLAMPSIA

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IT IS SOME twenty years since Hofbauer (1, 2) first postulated a 'pituitary-adrenal' mechanism as the cause of eclampsia. The proposal was attractive, although it was not supported at the time by direct experimental evidence. Many of the changes in pathological physiology which are characteristic of eclampsia, particularly water retention, oliguria and convulsions, are simulated by experimental pituitrin intoxication. However, crucial evidence that would incriminate the posterior pituitary in the etiology of eclampsia would depend upon the demonstration of pituitrin in the tissues or body fluids either in abnormal amounts or under conditions in which it is not otherwise found.

In 1931, Anselmino and Hoffman (3) reported that they had obtained a pituitrin-like substance in ultrafiltrates from the acidulated plasma of eclamptic patients. The identification of this supposed substance with pituitrin was based principally upon the observation that the ultrafiltrates caused inhibition of water diuresis in rabbits. However, in several severe cases of eclampsia, they also described a pressor effect. From their experiments they estimated that the plasma of eclamptic patients contained from 3 to 10 I.U. of pituitrin per liter. These experiments appeared to offer the first crucial proof of a relationship between pituitrin and eclampsia.

However, repetition of their experiments in other laboratories failed to confirm their findings. Indeed, Theobald (4) was unable to pass detectable amounts of known pituitrin through an acetic-collodion membrane prepared according to the specifications of Anselmino and Hoffman. From his experiments he concluded that such slight inhibition of water diuresis as results from such acetic-collodion membrane ultrafiltrates is not the result of pituitrin.

Levitt (5) was able to demonstrate an antidiuretic effect from ultrafiltrates prepared from dog blood to which pituitrin had been added in vitro, provided that the proteins were denatured and precipitated by acid and heat prior to filtration. How-

ever, only a small proportion of the original antidiuretic effect could be recovered by his method. If large doses of pituitrin (15 u) were injected intravenously into dogs, similarly prepared filtrates from blood withdrawn during the first few minutes after injection were antidiuretic. No effect could be demonstrated from blood withdrawn after 12 minutes. Thus, in dogs at least, exogenous pituitrin is rapidly inactivated by the blood and tissues.

Levitt tested ultrafiltrates similarly prepared from the blood of hypertensive and pre-eclamptic patients. No inhibition of water diuresis was encountered. However, ultrafiltrates from the blood of 4 severely eclamptic patients were all slightly antidiuretic, although the chloride content of the urine was not increased. He did not regard the antidiuretic effects from the blood of these 4 patients as sufficient to justify the conclusion that pituitrin had been demonstrated.

From additional evidence presented by Theobald, Jones and Schlapp (6), Heller and Urban (7) and Melville (8), it appears unlikely that the antidiuretic factor of the posterior pituitary could be actively present in the plasma in such quantities as Anselmino and Hoffman estimated. Even when large doses of pituitrin are injected intravenously into animals, significant amounts are demonstrable in the blood for relatively brief periods. Thus, Jones and Schlapp reported that after the intravenous injection of pituitrin, 85 per cent of the activity was lost from the blood within 20 minutes. They also found that 30 per cent of the injected pituitrin was recoverable from the urine within 2 hours. From other experiments of these workers, as well as from those of Heller and Urban, it appears that certain tissues, particularly liver, both in vitro and in vivo, have the property of rapidly inactivating posterior lobe extracts. In part, this effect would seem to be due to adsorption; for if pituitrin is added to cell-free aqueous or glycerine extracts of liver, it is inactivated. However, after heating, much of the activity returns.

Melville, using the bladder fistula dog as a test animal, has reported a method for extracting the antidiuretic principle from blood based upon the solubility of pituitrin in acid alcohol. He reports that 5 and 30 minutes after the injection into dogs of one unit of pituitrin per kilo of body weight, as little as 1 cc. of blood gives an antidiuretic response. Judging from the results reported, recovery of the antidiuretic effect by this method is more complete than by the method of Levitt. However, Melville also points out that after such injection of pituitrin, the antidiuretic effect rapidly disappears from the blood. Assays by his method of the antidiuretic potency of normal dog blood showed that 50 cc. produced an effect approximately equivalent to 5 milliunits of pituitrin. In men and non-pregnant women, smaller amounts of blood produced definite antidiuretic responses. The minimum amount of human blood which consistently showed the effect was 15 to 25 cc.

Tests were made using from 8 to 20 cc. of blood taken from 12 women during the latter part of pregnancy. Six of them had systolic blood pressures below 140 mm. Hg. The blood from 5 of these 6 normal patients gave no antidiuretic response. Of the 6 patients with systolic blood pressures over 140 mm. Hg, the blood from 5 showed some antidiuretic effect. The most pronounced effect (inhibition of diuresis for 54 minutes) was obtained from 8 cc. of blood taken from a patient with fairly marked hypertension, albuminuria and edema.

Although the number of patients investigated during pregnancy was too small to justify any definite conclusions, Melville's results fit in with others to suggest that during normal pregnancy the blood may contain even less actively antidiuretic substance than in the non-pregnant state. Since no blood was obtained from severely pre-eclamptic or eclamptic patients, whether a significant antidiuretic effect might

be demonstrated in their blood by his method remains unknown. In résumé, we are left with an attractive hypothesis as to the posterior pituitary and its rôle in the production of eclampsia unsupported by direct or crucial experimental evidence.

Recently, Gilman and Goodman (9) have presented new and convincing evidence that the posterior hypophysis serves as at least one important physiologic regulator of the renal excretion of water. They showed that a substance which inhibits water diuresis in rats may be recovered from the urine of dehydrated normal rats but not from the urine of dehydrated hypophysectomized rats. The latter are peculiarly susceptible to dehydration and die after 36 to 48 hours of water deprivation. Moreover, chemical treatment which destroys the antidiuretic effect of known pituitrin (hydrolysis in 2N NaOH or 1 per cent HCl for 30 minutes) likewise destroys the similar effect of preparations from the urine of dehydrated animals.

According to Gilman and Goodman, the pituitrin-like substance is recoverable from the urine after dehydration accomplished either by withholding water for 24 to 96 hours, or after giving hypertonic NaCl solution by mouth. No antidiuretic effect was obtained from urine under usual conditions of water and food intake or after ingestion of large amounts of water. The minimum period of withholding water before significant amounts of antidiuretic substance were demonstrable was approximately 24 hours. The amount recoverable increases up to 48 hours when it becomes maximal. From their bioassays, they have calculated that 4 rats under these conditions of water deprivation will excrete approximately 1 I.U. of pituitrin in 72 hours.

These findings have offered a new approach to the investigation of possible relationships between pituitrin and water retention in pregnancy toxemias, for if pituitrin acts upon the kidney in sufficient amounts to account for the oliguria and water retention, it might well be demonstrable in the urine. We have approached this problem using essentially the technique described by Gilman and Goodman. Unimportant modifications have been made in the technique for the preparation of the urine concentrates and for the bioassay of the antidiuretic effect to suit our purposes.

METHODS AND OBSERVATIONS

Preparation of urine concentrates. Urine is collected free of fecal contamination and acidulated with 2 per cent acetic acid. Electrolytes and urea are reduced to negligible amounts by dialysis of the urine against running water for 3 hours. A cellophane membrane of 0.00072 in. thickness has been used which is not permeable to known posterior pituitary antidiuretic principle under the conditions of these experiments. After dialysis, the urine is concentrated sufficiently that the amount to be injected into each animal lies between 0.5 and 1.0 cc.

In the preparation of concentrates from the urine of patients with preeclampsia and eclampsia, the added problem of heavy proteinuria presented itself. In most of our experiments, we have deproteinized the acidulated urine by heating in a water bath to 80° and centrifugation before dialysis and concentration. The amount of urine represented in the concentrate which we have given to each animal has ranged between 3 and 12 cc.

Bioassay of the antidiuretic effect. In the bioassay of the antidiuretic effect, groups of 4 adult male rats on a standard ration are used. Food, but not water,

is withheld for 8 hours prior to the experiment. In the early experiments, we gave a standard dose of water by gavage, equivalent to 5 per cent of the body weight and at the same time injected subcutaneously the urine concentrate to be tested. The percentage of ingested water excreted was plotted against the time in 15-minute intervals for 3 or more hours. The excretion curves of the experimental group were then compared with those of controls which had received water alone, as well as with comparable subcutaneous doses of water and standard doses of posterior pituitary extract. This method of bioassay gives a fair quantitative response for antidiuretic effects with doses of 5 to 50 milliunits of posterior lobe extract per animal.

More recently, we have given two doses of water by gavage, equivalent to 5 and 3 per cent of the body weight respectively, one hour apart. The concentrate to be tested is given intramuscularly when a good diuresis has become established. This is usually 80 minutes after the first gavage. This latter method offers the additional check that an established diuresis is inhibited by the antidiuretic substance and subsequently recurs. In these latter experiments, we have used as the amount of water to be excreted the total of the two doses less the amount excreted at the time of injection. This amount is usually about 6.5 per cent of the body weight of the animals. For this reason, it will be noted that the time at which 50 per cent excretion is reached is somewhat greater than Gilman and Goodman's standard.

Control experiments. We have repeated the experiments of Gilman and Goodman, with the exception of the experiments upon hypophysectomized rats. Our results are entirely in accord with their findings. After dehydration for from 24 to 72 hours, concentrates prepared from the urine of normal male rats are markedly antidiuretic. Similar antidiuretic effects were obtained from the urine after giving 5 per cent NaCl solution by gavage, equivalent to 5 per cent of the body weight.

Bioassay of known amounts of pituitrin gave reasonably good correlation between dose and response. Known amounts of pituitrin when added to urine were recoverable by the procedure we have outlined. A typical experiment with 10 and 20 μU of pituitrin per animal is shown in figure 1. Hydrolysis of potent antidiuretic urine concentrates from dehydrated rats with 2N NaOH for 30 minutes destroyed the antidiuretic effect.

The primary questions to be answered in this investigation were two: First, under what conditions, if any, do women during normal pregnancy excrete an antidiuretic substance in the urine? Second, do patients with preeclampsia and eclampsia excrete an antidiuretic substance in the urine; if so, is it to be found in greater amounts or under different conditions than in normal pregnancy?

Observations during normal pregnancy. Urine specimens were obtained from 22 patients during the last trimester of pregnancy. Most of them were morning specimens, but all subjects were on voluntary water intakes. Markedly dilute specimens were discarded, the specific gravities of the specimens used varying from 1.018 to 1.035. Concentrates were prepared representing from 4 to 12 cc. of urine per animal. In 19 instances, no inhibition

of water diuresis was encountered. There was an equivocal response from one specimen, comparable to that from 5 μU of pituitrin. The other two concentrates evoked antidiuretic responses comparable to the effect from 10μU of pituitrin per animal. It is perhaps significant that one of these two patients had gained 9 pounds in the past week, although there was neither hypertension nor albuminuria (table 1).

Urine was obtained at the time of delivery from 8 normal patients. None of these urines showed an antidiuretic effect, although the specific gravities

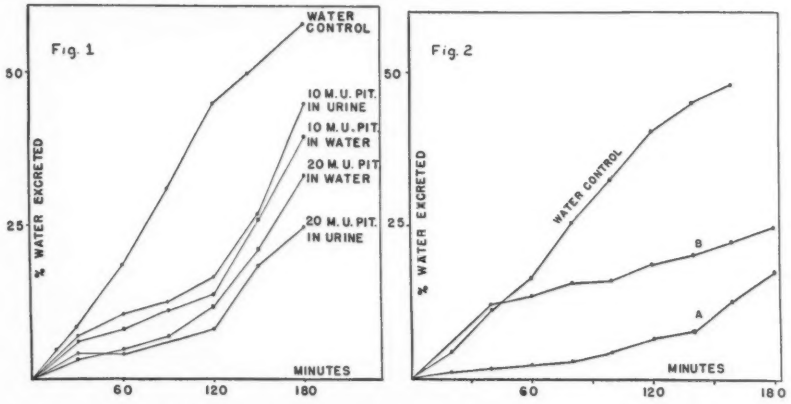

Fig. 1. Comparison between the antidiuretic responses to the intramuscular injection of, *a*), aqueous solutions of 10 and 20 milliunits of pituitrin per animal and, *b*) the same amounts of pituitrin added to 50 cc. of urine which was then dialysed and concentrated by the method described. This experiment is of the second type discussed; the injections having been given after diuresis was established. Fig. 2. The two normal women from whose urines these concentrates were prepared had been dehydrated by first restricting the daily fluid intake to 800 cc. for 2 days and then withholding all fluids for 24 and 18 hr. respectively. The last specimen voided was used. A, A normal multipara in the last month of pregnancy. B, A non-pregnant subject. This experiment is of the second type discussed in which water diuresis had been established in the animals before the injection was given.

all exceeded 1.020. Postpartum urines from 13 patients were examined at varying intervals after delivery from a few hours to two weeks. An antidiuretic effect was observed comparable to approximately 15μU of pituitrin per animal from one specimen collected 6 hours postpartum. However, this patient had received 20U of pituitrin intramuscularly at the time of delivery.

We interpret the results of these 43 experiments as indicating that under conditions of voluntary water intake, little or no antidiuretic substance is demonstrable in these amounts of urine for normal women during the last trimester of pregnancy, at the time of delivery or in the early puerperium.

When normal women were sufficiently dehydrated by withholding water,[1] the urine exhibited markedly antidiuretic effects. This was found to be true both of non-pregnant women and of women in the last trimester of pregnancy (fig. 2).

[1] The fluid intake was limited to 800 cc. per day for 2 days, and salt was permitted ad lib. All fluids were withheld subsequently for 18 to 24 hours, and the last urine specimen was used.

Observations upon toxemic patients. In testing the urine from patients with so-called toxemias of pregnancy, it was important to avoid any confusion which might result from specimens obtained after therapy, and particularly dehydration therapy, had been begun. For this reason, when possible, the specimens examined were collected upon admission to the hospital or shortly thereafter. Subsequently, specimens were examined only from patients without sharply restricted intakes.

In table 2, the antidiuretic responses which resulted from concentrates prepared from 28 urine specimens from toxemic patients are recorded. The

TABLE 1. ANTIDIURETIC RESPONSES TO URINE CONCENTRATES FROM NORMALLY PREGNANT WOMEN

Sp. Gr. of Urine	Conc. Equiv. in cc. of Urine Per Animal	Antidiuretic Response in μU of Pituitrin	Remarks
1.025	12	None	
1.028	12	None	Edema of legs
1.022	12	None	
1.022	12	None	
1.022	12	None	
1.022	12	None	
1.025	12	None	
1.020	12	None	
1.028	10	None	
1.024	10	None	
1.018	8	None	Edema of legs
1.025	8	None	
1.035	8	None	Mod. edema of legs
1.018	8	None	
1.028	7	None	
1.018	6	None	
1.022	6	None	
1.025	4	None	
1.027	4	None	
1.029	12	5	No edema
1.024	12	10	Slt. edema
1.025	12	10	9 lb. wt. gain past wk.

These concentrates were prepared from the urines of 22 patients during the last trimester of pregnancy. None had instructions as to water and salt intake. None had hypertension or albuminuria.

so-called 'toxemias' ranged from mild asymptomatic hypertension without albuminuria or edema through severe clinical pre-eclampsia and eclampsia. It will be observed that in 11 of the 28 cases, the urine concentrate caused either no effect or an equivocal one, but that in 8 of the 17 remaining instances in which a definite antidiuresis resulted, the antidiuretic response was comparable to 20μU or more of pituitrin per animal. It is also of interest that 7 of these 8 patients had clinical edema, whereas only 2 of the 11 who showed no antidiuretic response had edema.

It has been our impression that the presence of antidiuretic substance in the urine is much more closely correlated with the state and phase of acute water retention, as indicated by rapid weight gain, edema and oliguria than with the elevation of the blood pressure or the degree of albuminuria.

In figure 3, the marked antidiuretic response from the urine of a severely pre-eclamptic patient is shown. The antidiuretic effect disappears shortly

after delivery as is shown by the lack of effect of concentrates prepared from urine during the first and second weeks postpartum.

According to the well supported thesis of Gilman and Goodman, it is the physiologic rôle of pituitrin to appear in the blood and urine under conditions of water balance which necessitate the tubular re-absorption of water. This state of affairs comes into existence under physiologic conditions when either the water intake is deficient or the intake of salt is excessive. They were able to demonstrate the antidiuretic substance in the urine of rats

TABLE 2. ANTIDIURETIC RESPONSES TO URINE CONCENTRATES FROM 28 PREGNANT WOMEN WITH VARYING DEGREES OF HYPERTENSION, ALBUMINURIA AND EDEMA

B. P.	Alb.	Edema	Sp. Gr.	Conc. Equiv. in cc. of Urine Per Animal	Antidiuretic Response in μu of Pituitrin	Remarks
140/100	1	0	1.032	3	None	No symptoms
146/88	2	2	1.026	3	None	No symptoms
148/106	1	0	1.028	3	None	No symptoms
154/88	1	0	?	3	None	No symptoms
154/100	3	0	1.024	3	None	No symptoms
160/110	1	0	1.031	12	None	No symptoms
190/110	2	2	1.032	12	None	No symptoms
170/104	0	0	1.014	4	None	No symptoms
205/120	3	0	1.027	4	None	No symptoms
204/120	3	0	1.020	4	<5	No symptoms
200/110	3	0	1.028	4	<5	No symptoms
140/90	0	?	1.015	3	5	No symptoms
170/100	4+	2	?	5	5	No symptoms
170/100	2	1	?	4	5	No symptoms
160/110	3	1	1.029	12	5	No symptoms
174/112	2	4	?	3	5–10	No symptoms
140/100	3	0	1.024	3	10	No symptoms
170/100	4+	2	1.024	3	10	Clinical pre-eclampsia
168/112	1	3	?	4	10	No symptoms
140/90	1	4	1.020	3	10	No symptoms
144/96	2	0	1.035	3	20	No symptoms
140/90	3	4	1.034	12	20	No symptoms
170/110	4+	4	1.036	12	>50	Clinical pre-eclampsia
210/120	4+	4	1.034	5	>50	Clinical pre-eclampsia
200/130	4+	4	1.040	3	>30	Clinical pre-eclampsia
196/120	4+	4	1.026	3	>30	Clinical pre-eclampsia
180/120	4+	2	1.027	8	>50	Eclampsia
170/115	4+	2	1.021	7	>50	Eclampsia

under both of these conditions. Conversely, when the water intake is excessive, and there is no necessity for tubular re-absorption of water, pituitrin disappears and water diuresis ensues. The antidiuretic principle was never recovered from the urine during water diuresis.

It seemed desirable, in order more definitely to establish the abnormality of the appearance of the antidiuretic substance in the urine of pre-eclamptics and eclamptics, to test urine concentrates collected after excesses of water by mouth. An eclamptic patient who had had three convulsions was given 1400 cc. of water by mouth, and the urine was collected by catheter 3 hours later. In spite of water, the specific gravity of the 170 cc. of urine excreted was still 1.021, and concentrates prepared from it were actively antidiuretic.

One portion of the urine from this patient with eclampsia was dialysed and concentrated without preliminary deproteinization. The concentrate was divided into two parts. The first was injected unaltered. The second was slightly acidulated, heated to 80° and centrifuged, the supernatant fluid being injected. It was of considerable interest that the protein-laden concentrate did not inhibit diuresis, whereas the supernatant fluid from the other portion, after denaturation and precipitation of the protein, was active (fig. 4). Re-extraction of the protein precipitate resulted in approximately half the antidiuretic effect of the first supernatant fluid. This observation

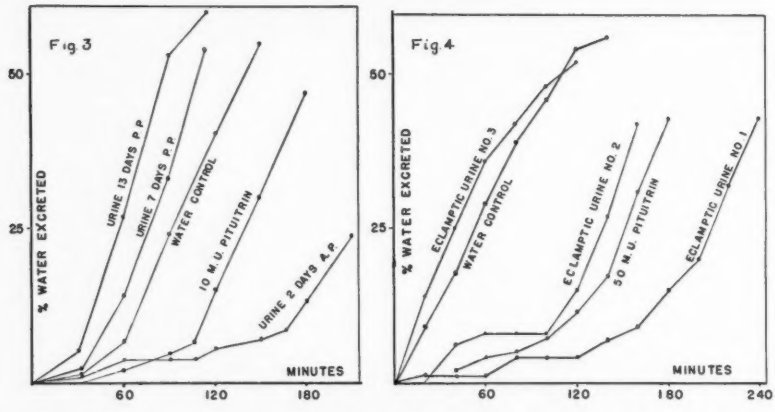

Fig. 3. Three concentrates were prepared from the urine of a severely pre-eclamptic primigravida with twins. One specimen was prepared from urine collected 2 days antepartum; the other two were from urine collected 7 and 13 days postpartum respectively. For comparison, the response to 10 milliunits of pituitrin per animal is shown. This experiment is of the first type discussed. A single dose of water was given, and the urine concentrates were injected at the time of the gavage. Fig. 4. This figure shows the antidiuretic responses to concentrates prepared from the urine of an eclamptic patient who had been given 1400 cc. of water 3 hours previously. No. 1 shows the response to the usual type of concentrate. No. 2 shows the response from a second concentrate which was deproteinized after dialysis and concentration. No. 3 shows the lack of antidiuretic effect which resulted from injection of the protein laden concentrate from which No. 2 was made. The response to 50 milliunits of pituitrin in this experiment was less than usually obtained. This experiment is of the second type discussed in which diuresis had been established before injection of the concentrates.

suggests that the protein in pre-eclamptic and eclamptic urine may adsorb and inactivate the antidiuretic factor, as blood serum, liver and liver extracts have been shown to adsorb known pituitrin.

In several previously normal patients with rapidly progressing hypertension and albuminuria late in pregnancy, we have been unable to demonstrate an antidiuretic effect from the urine, even though several specimens have been tested. Recently, a primigravida some 4 weeks from term developed marked hypertension and albuminuria over a few days while under observation in an institution. There was little clinical evidence of edema and no marked weight gain. Although the urine output of this patient after drinking a liter of water was only 210 cc. in 5 hours, and the specific gravity of the urine was above 1.020, we were unable to detect an antidiuretic effect from specimens collected either before or after the administration of water. This

patient showed little disturbance in water balance in so far as unexplained weight gain is concerned and was entirely free from symptoms of pre-eclampsia. However, following delivery, marked oliguria developed. The urine secreted between 6 and 12 hours postpartum was 75 cc. A concentrate from this specimen showed a definite antidiuretic effect.

In view of the observations of Thorn, Nelson and Thorn (10) that the estrogens and related sterols cause water retention in dogs; that they un-

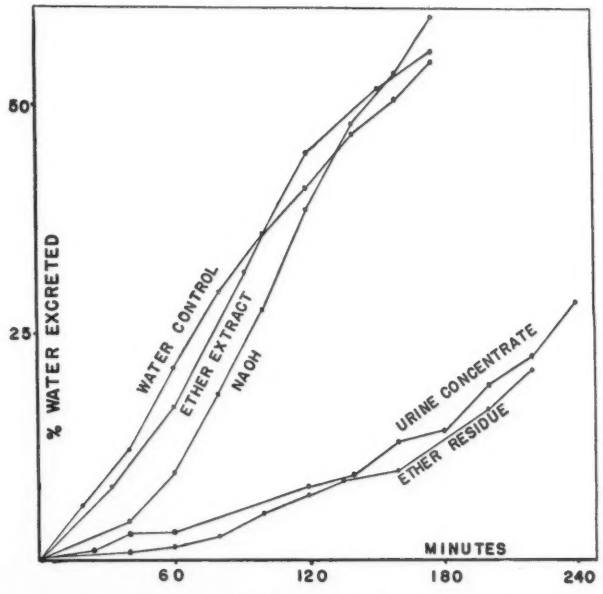

Fig. 5. The concentrate prepared from 120 cc. of urine from a severely pre-eclamptic patient was divided into 3 parts. One was injected unaltered. The second was hydrolysed with NaOH and injected. The third was extracted three times with ether. Both the pooled ether extracts and the ether-insoluble residue were tested. This experiment is of the second type in which diuresis had become established before injection of the concentrates.

doubtedly are operative in the appearance of premenstrual edema in women,[2] and the common knowledge that the blood and urine of women during pregnancy contain large amounts of estrogens, it appeared desirable to learn whether the antidiuretic effect from pre-eclamptic and eclamptic urine might be due to one of the estrogens. An actively antidiuretic concentrate from the urine of a severely pre-eclamptic patient was extracted 3 times with ether. The ether extracts were pooled and tested, as was the ether-insoluble fraction. As will be seen from figure 5, the ether-soluble fraction was inert, and the ether-insoluble fraction was antidiuretic.

[2] In a single experiment, we have obtained marked antidiuretic activity in a concentrate from the urine of a patient with marked premenstrual edema.

In the experiment illustrated in figure 5, a third portion of the urine concentrate was hydrolysed with NaOH with resultant inactivation of the antidiuretic effect. As has been pointed out by Gilman and Goodman, such treatment also destroys the antidiuretic effect of known pituitrin.

DISCUSSION

These studies indicate that at least some patients with pre-eclampsia and eclampsia excrete an antidiuretic substance in considerable quantities in the urine under conditions of fluid intake in which normally pregnant patients excrete little or none. The antidiuretic substance recovered from the urine of these pre-elcamptic and eclamptic patients resembles in certain respects the antidiuretic principle of the posterior pituitary. When dialysed against running water, it is retained in a cellophane membrane which also retains known pituitrin. It is destroyed by hydrolysis with NaOH which also destroys known pituitrin. It is insoluble in ether and, therefore, probably is not an estrogen. Preliminary experiments suggest that it may be adsorbed and inactivated by the protein in concentrated eclamptic urine, and like known pituitrin, may be recovered in the supernatant fluid after denaturing and precipitating the protein. In these respects, then, the antidiuretic substance in pre-eclamptic and eclamptic urine resembles that of the posterior pituitary.

A similar antidiuretic substance is recoverable from the urine of normal patients during the last trimester of pregnancy in at all comparable amounts only if they are markedly dehydrated. In this respect, the normally pregnant woman responds to dehydration as do the experimental animals described by Gilman and Goodman. It would seem not improbable that the antidiuretic principle recovered from the urine has its origin in the posterior pituitary in all three instances. However, this substance appears in the urine of pre-eclamptic and eclamptic patients in excessive amounts and under conditions of water intake in which it is not found, at least in significant amounts, during normal pregnancy in women or in non-pregnant animals.

We would emphasize again that the antidiuretic effect has not been obtained from the urine of every pregnant woman with elevation of the blood pressure or albuminuria. Its presence in the urine has been much better correlated with water retention as indicated clinically by sudden weight gain, edema and oliguria. It disappears with the loss of weight and diuresis which accompany clinical improvement.

We have not yet investigated extensively the urine from patients in whom sudden unexplained weight gain, edema and oliguria were not yet accompanied by hypertension and albuminuria. However, it would not be surprising if significant amounts were demonstrable in the urine of such patients before the appearance of hypertension and albuminuria. One of our three so-called normal patients whose urine showed a slight antidiuretic effect had gained 9 lb. in the preceding week.

We would also point out that in several patients with pre-eclampsia, specimens collected from day to day sometimes showed an antidiuretic re-

sponse on one day and none the next. This is not surprising, since the clinical disease is often characterized by improvement and exacerbation over brief periods.

The water retention of pre-eclampsia and eclampsia could be explained upon a basis of either *a*) increased pituitrin secretion or *b*), decreased ability of the tissues, and particularly the liver, to inactivate or destroy it. There is evidence from several sources that certain of the physiologic effects of posterior pituitary extracts are inactivated when such extracts are mixed in vitro with the blood or serum from normally pregnant women. The blood or serum from non-pregnant women is much less effective in this respect. The ability of pituitary extracts to expand melanophores in amphibia is markedly inhibited by mixing the extracts with the serum of women late in pregnancy (11). Serum from women late in pregnancy exerts an inhibitory influence upon pituitrin many times as great as that from non-pregnant women (11, 12, 13). Kustner has reported that this inhibitory effect on pituitrin is conspicuously absent from the serum of eclamptics. Moreover, normally pregnant women are much more tolerant to exogenous pituitrin injected intravenously (3 to 5U) than are non-pregnant women. The pressor effect is less, and subjective symptoms of discomfort are considerably less in the pregnant group (14).

Although normal women are relatively tolerant to small doses of pituitrin (1 to 2U subcutaneously) during the latter months of pregnancy, patients with pre-eclampsia are very sensitive to it and respond with marked and relatively prolonged rise in blood pressure, prolonged oliguria and often with increase in characteristic pre-eclamptic symptoms, Dieckmann and Michel (15) have demonstrated that this sensitivity to pituitrin is sufficiently characteristic of pre-eclampsia to be of aid in differential diagnosis between pre-eclampsia and chronic vascular and renal diseases complicated by pregnancy.

One possible explanation for this hypersensitivity of pre-eclamptic and eclamptic patients to exogenous pituitrin might well be decreased ability of the tissues (liver) and blood to fix or inactivate it. Whether the mechanism of water retention in these patients is increased secretion or decreased ability of the tissues to inactivate pituitrin, the effect upon the kidneys might well be the same. In either instance, what rationale would there be for the sharp restriction of water commonly practiced? Dehydration is an effective physiologic means of stimulating the secretion of the antidiuretic principle. Restriction of salt, on the other hand, would have more rationale, for the less salt there is to be excreted by the kidney, the less necessity for the tubular re-absorption of water, and hence, the less stimulation to posterior pituitary activity. It would appear, if one were willing to accept these physiologic tenets, that moderate fluid and low salt intakes were desiderata. This is precisely the conclusion which de Snoo (16) has reached on purely clinical grounds.

The fact that the antidiuretic substance in the urine of pre-eclamptic and eclamptic patients is insoluble in ether and therefore not an ordinary estrogen is of interest in view of the studies of Thorn and collaborators on

the origin of premenstrual edema. The experimentally produced water retention described by them from the injection of estrogens into dogs appears and disappears over a period of several days. In our hands, water diuresis in rats is not inhibited by the injection of estrone and estriol in the acute experiment. It would seem possible that the effect described by Thorn et al. might be due to posterior pituitary stimulation which results from the estrogens and related sterols. Such a mechanism might also be operative in pre-eclampsia and eclampsia, although it is reported that the total excretion of estrogens is diminished in these conditions.

The experiments of McManus, Riley and Janney (17) which demonstrated that as normal pregnancy advances, the diuresis induced by standard doses of water becomes more and more delayed, and that patients with pre-eclampsia and eclampsia show marked exaggeration of this impaired ability to excrete water are entirely in consonance with our results. Although urine concentrates from only 3 of 30 patients normally pregnant showed a slight antidiuretic effect, it is entirely possible that if greater quantities of urine were concentrated, small amounts of the antidiuretic principle could be demonstrated during the later months of pregnancy and particularly at the time of delivery. If pituitrin is a factor in the onset of labor, it is possible that it might be detected in concentrates from larger amounts of urine.

It will be noticed from figure 3 that following the injection of a concentrate from the postpartum urine of a pre-eclamptic patient, the rate of urine excretion was considerably greater than in the control experiment. We have observed this exaggerated diuresis after a number of postpartum urine concentrates. Active diuresis is characteristic of the early puerperium, and this observation raises the question whether there may be an actively diuretic factor operative, rather than simple withdrawal of the pituitrin effect. It has been demonstrated by one of us (18) and amply confirmed by others (19, 20, 21) that certain anterior hypophyseal extracts exert a prolonged and powerful diuretic effect in dogs. The diuretic effect seems to be associated with the thyrotropic hormone, for first, maximal diuresis results from extracts which also evoke good thyrotropic responses, and second, no diuresis results from such extracts after thyroidectomy. However, that the effect is primarily hypophyseal is indicated by the fact that after giving the thyroidectomized animal thyroxin, the diuretic response can again be produced by such anterior hypophyseal extracts.

Additional evidence that the anterior hypophysis elaborates an actively diuretic principle lies in the observation that prolonged or permanent diabetes insipidus of marked degree results from posterior lobe removal, only provided that the anterior hypophysis or a part of it remains intact.

Bonilla and Kramann (22) have recently reported the demonstration of thyrotropic hormone in the urine of patients with hyperemesis gravidarum and pre-eclampsia. They state that there is no correlation between the severity of the disease and the thyrotropic response of the urine extracts. No such effects were obtained from similarly prepared urine extracts from

non-pregnant, normally pregnant and puerperal women. These workers were not concerned with water metabolism, but suggested that the elaboration of the thyrotropic hormone was a defense mechanism to the posterior lobe activity in pre-eclampsia.

In both hyperemesis and pre-eclampsia, the kidney secretes a concentrated urine. In the former instance, this is because of dehydration, and in the latter perhaps because of the abnormal action of pituitrin or a pituitrin-like antidiuretic substance upon the kidney. This would suggest that in each instance the antidiuretic effect may be counteracted by the actively diuretic effect of the thyrotropic hormone. We would anticipate that urine collected during the phase of oliguria and water retention in pre-eclampsia would show little or no thyrotropic effect and that with a phase of diuresis the latter would appear.

On the basis of these observations, we are unwilling to exclude a balance between anterior and posterior hypophyseal diuretic and antidiuretic functions respectively in the control of water balance in pre-eclampsia and eclampsia. We cannot apply without reservations the concept of Gilman and Goodman[3] that regulation of the renal excretion of water is a function of the posterior lobe alone. Indeed, were a commercial anterior lobe diuretic extract available, in our opinion, it should warrant clinical trial in pre-eclampsia. However, none of the sterile commercial preparations which have been tried on animals have produced a diuresis in dogs at all comparable to that obtainable from more crudely but freshly prepared extracts.

We are of the opinion that either the abnormal secretion of an antidiuretic substance which we are as yet unable to distinguish from pituitrin, or possibly the inability of the tissues to inactivate usual amounts of this substance is the immediate explanation for the water retention of pre-eclampsia and eclampsia. Whether deficiency of an anterior hypophyseal antagonist to the antidiuretic effect of the presumptive pituitrin is a factor offers ground for further experiments.

Pharmacologically, it requires some 200 times as much pituitrin to elicit the pressor effect as to inhibit diuresis. Failure to demonstrate pituitrin in the blood of eclamptic patients in considerable amounts speaks against excess pituitrin as the direct cause of the hypertension, although the lack of a pituitrin inhibitory substance in the serum and tissues which has been demonstrated to exist in normal pregnancy might materially alter the sensitivity of the smaller vessels to smaller amounts. Since water retention usually precedes the hypertension and albuminuria, it would seem that another possible factor in the etiology of the hypertension might lie in secondary effects of pituitrin upon the kidney, for it has been demonstrated that an injured ischaemic kidney may cause hypertension and albuminuria.

[3] 'In our opinion all levels of renal activity with respect to water excretion can be explained by the relative efficiency of the tubules in the reabsorption of water. Thus, it is logical to assume that just as dehydration stimulates the posterior hypophysis to increased secretary activity, hydration suppresses activity and allows for the destruction of the hormone already present in the blood stream.'

SUMMARY

Concentrates prepared from the urine of pre-eclamptic and eclamptic patients during the phase of acute water retention exert marked antidiuretic effects upon water diuresis in rats. This effect persisted in concentrates, prepared from the urine of an eclamptic patient, collected after giving 1400 cc. of water by mouth.

Similarly prepared concentrates from the urine of normally pregnant patients during the last trimester of pregnancy and at the time of delivery exert little or no antidiuretic effect. However, if normally pregnant patients are sufficiently dehydrated by withholding water, the urine contains amounts of antidiuretic substance comparable to those found in pre-eclamptic and eclamptic patients who are on voluntary or forced fluid intakes.

The antidiuretic principle recovered from the urine of pre-eclamptic and eclamptic women resembles pituitrin. This pituitrin-like substance acting upon the kidney in abnormal amounts, or perhaps inadequately opposed by an antagonist, offers an immediate mechanism for the water retention of pre-eclampsia and eclampsia.

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HORMONAL INFLUENCES ON THE EXCITABILITY OF THE MUSCULAR PROTOPLASM OF THE RAT'S UTERUS

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ONE OF US, (1), has previously reported two types of response to electrical stimulation, observed in the rat's uterus in vitro. These types differed greatly in magnitude and at that time were assumed to correspond with the 'maximal' and 'submaximal' contractions described by Harne (2) as being characteristic of the spontaneous activity of the rat's uterus in vitro. It was found that the electrical excitability (chronaxie) of the submaximal contractions varied in a cyclic manner coincident with the estrous cycle of the rat, while the excitability of the maximal contractions underwent little or no change. It seemed apparent that the submaximal contractions were reflecting the hormonal influences responsible for the estrous cycle and it became of interest to discover the particular hormones which were operating in this regard.

In the course of the present work, it soon became apparent that the minimal responses to electrical stimulation, which vary with the estrous cycle, are not analagous to the submaximal spontaneous uterine contractions of Harne. It will therefore be best to use a distinctive nomenclature from the outset. The terms 'submaximal' and 'maximal contractions' will be applied to spontaneous uterine activities only, while the reactions to electrical stimulation will be called 'minimal' and 'major responses.'

METHODS AND RESULTS

The apparatus and methods employed were the same as those previously described (1) except for the manner in which the horn of the uterus was suspended and its contractions recorded (fig. 1). At a point midway between its two ends, the uterine horn was fixed to a glass rod by a fine thread passed through the parametrium, so as not to disturb the uterine muscle. The free vaginal and ovarian ends were connected by threads to recording levers, so that the uterine horn was suspended in the form of a 'V.' A third recording lever was then connected with the other two in such a way as to summate their movements. It was thus possible to record simultaneously, in an intact uterine horn, the respective contractions of the vaginal end, of the ovarian end, and of the entire organ.

[1] Aided by the A. B. Kuppenheimer Fund.

The Difference Between Spontaneous Uterine Contractions and the Responses to Electrical Stimulation

With the aid of our mechanical arrangement it was readily seen that the uterus does not contract simultaneously throughout its length. Spontaneous contractions almost always start in the ovarian end of the horn.[2] The wave of contraction travels towards the vaginal end until the whole organ is contracted. The first relaxation is seen at the point where contraction had

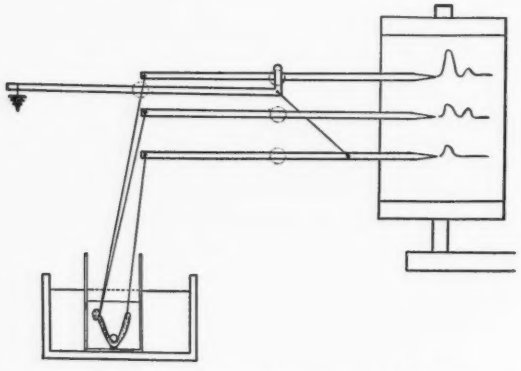

Fig. 1. LEVER ARRANGEMENT FOR RECORDING, SIMULTANEOUSLY BUT SEPARATELY, THE MOVEMENTS OF EACH END OF THE UTERINE HORN AND THE ENTIRE HORN RESPECTIVELY. The uppermost lever summates the movements of the other two.

started, and follows a similar course, until the entire organ is relaxed. This agrees with the observations made through an abdominal window in vivo, by Kehrer (3).

As is illustrated in figure 2, the wave of contraction does not always travel the entire length of the horn. When it does, the separate contractions of the ovarian and vaginal end add up to a maximal contraction of the whole uterus. When it fails, a submaximal contraction of the whole uterus is recorded. Figure 2 also illustrates the greater activity of the ovarian as compared to the vaginal end, when the two portions of the uterine horn are studied separately, as noted by Kehrer (3) and Harne (4).

Electrical stimulation of the uterus may result in major responses entirely comparable to either the maximal or submaximal spontaneous contractions described above. These major responses are obtained when the stimulus is applied just before an expected spontaneous contraction, or at any time when the uterus is under the influence of pantopon (1). Under all other circumstances, however, electrical stimulation results in a minimal response, which consists of a local contraction of the muscle around the stimulating electrode, without any sign of a contraction wave (fig. 3).

In these experiments, as in our previous ones, the positive and negative

[2] During estrus and pregnancy, and after the exhibition of pantopon, there is an increase in the number of contractions beginning in the vaginal end, as compared to diestrus.

electrodes were applied at the opposite ends of the uterine horn. Our results apparently differed from those obtained on other muscle and nerve preparations in that excitation always occurred at the anode on making the connection (fig. 3).

Comment. It is evident that, contrary to our previous assumption (1) the minimal responses to electrical stimulation are not analagous to any spontaneous contraction. Maximal spontaneous contractions are conducted

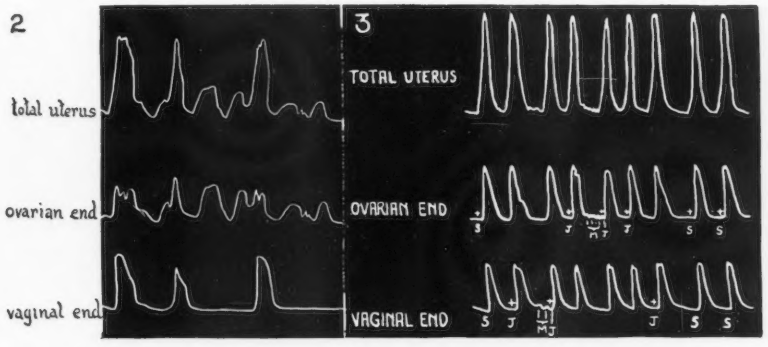

Fig. 2. A RECORD OF SPONTANEOUS CONTRACTIONS OF THE PROESTROUS UTERUS MADE WITH THE APPARATUS SHOWN IN FIG. 1. Note that the contractions begin in the ovarian end. Those contractions which are conducted through to the vaginal end, appear as maximal contractions on the total uterus record, while those which fail to reach the vaginal end appear as submaximal contractions on the total uterus record. Fig. 3. A RECORD OF SPONTANEOUS CONTRACTIONS AND OF RESPONSES TO ELECTRICAL STIMULI, IN AN ESTROUS UTERUS, MADE WITH THE APPARATUS SHOWN IN FIG. 1. All spontaneous contractions and the major responses to electrical stimulation are conducted along the entire uterine horn, in this phase of the estrous cycle; the minimal responses to electrical stimulation are not conducted. The plus sign indicates the end of the uterus in which a particular contraction was observed to begin. S = spontaneous contractions (all are maximal during estrus), J = major responses to electrical stimulation, M = minimal responses to electrical stimulation. Responses to electrical stimulation always started at the anode on making the connection. The end of the uterine horn to which the anode was applied is indicated by the placement of the identifying letter.

throughout the uterus; submaximal spontaneous contractions are conducted for shorter distances; major responses to electrical stimulation may resemble either of the foregoing; while minimal responses to electrical stimulation are not conducted at all. This suggests the possibility that only the muscular tissue and not the nervous elements are involved in the latter type of response. The following observations tend to support this conclusion.

Evidence that the Minimal Response to Electrical Stimulation is a Purely Muscular Reaction

Chronaxie. The major responses to electrical stimulation resemble the spontaneous activity of the neuro-muscular uterine complex, while the minimal responses do not. This difference is accompanied by a difference in chronaxie; the rheobase of the minimal response is greater and its chronaxie is longer than those of the major response (1).

Resistance to oxygen lack. Although it is not finally settled whether the

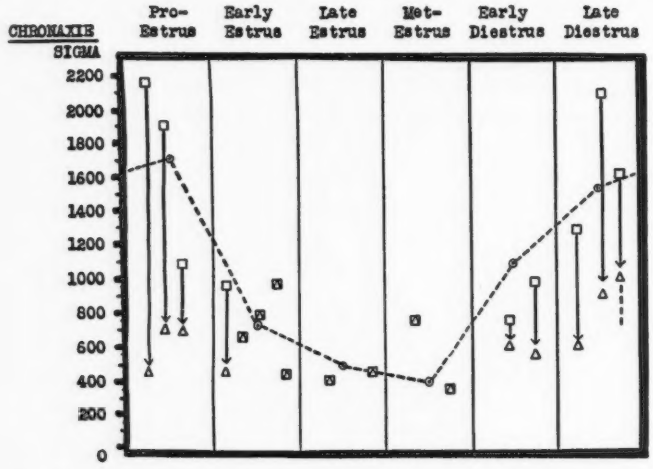

Fig. 4 □ Before pitocin; △ after pitocin; ⊠ no effect from pitocin

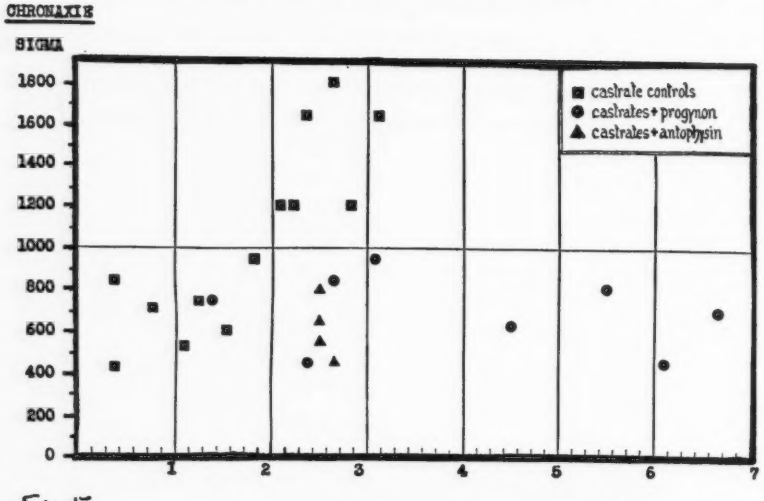

Fig. 5

Fig. 4. THE IN VITRO EFFECTS OF THE OXYTOCIC PRINCIPLE OF THE POSTERIOR PITUITARY GLAND, ON THE CHRONAXIE OF THE MINIMAL RESPONSES TO ELECTRICAL STIMULATION, IN UTERI REMOVED FROM NORMAL RATS AT VARIOUS TIMES IN THE ESTROUS CYCLE. The dotted line represents the average chronaxie values for untreated normal animals, throughout the estrous cycle, which we have previously reported. Each of the indicated phases of the estrous cycle occupies approximately one day. Fig. 5. CHRONAXIE OF THE MINIMAL RESPONSES TO ELECTRICAL STIMULATION IN UTERI REMOVED FROM RATS AT VARYING INTERVALS AFTER CASTRATION. The time scale is in weeks. Each square, circle or triangle represents a separate experiment on a different animal. In the experiments represented by the circles and triangles, estrogenic and APL substances respectively were administered to the castrate animals for a number of days preceding the removal of the uterus for the chronaxie determination.

initiation of spontaneous contractions in smooth muscle rests with the nervous or muscular elements, it seems fairly certain (from observations on the gut) that the spread of the contraction wave and the coordination of movements is a function of the nervous tissue (5, 6, 7). It is also known that the presence of oxygen is essential for nervous activity, while muscle can contract in an oxygen-free medium (5). Elicitation of contractions during oxygen lack therefore indicates a purely muscular response. Alvarez (9) has used this criterion in studying the gut.

We have shown that minimal responses to electrical stimulation can be obtained during lack of oxygen, at a time when spontaneous contractions have ceased and major responses are not obtainable (1).

Susceptibility to quinine intoxication. Quinine is toxic to protoplasm in general, but particularly to muscular protoplasm (8, 10). Concentrations of quinine which are toxic to muscle have no effect on nervous tissue. As regards the uterus, certain small doses increase uterine activity, while larger doses cause paralysis and irreversible damage (8, 11, 12). Both these effects are considered to result from a direct action upon the muscle only (8, 11). The demonstration that quinine influences the excitability of the minimal responses but not of the major responses to electrical stimulation, would therefore favor the view that the former represent purely muscular reactions.

We have found that quinine bisulphate in concentrations of 1:200,000 to 1:50,000, increases the chronaxie of the minimal responses during estrus or metestrus from an initial value of about 400σ to values as high as 1500σ.[3] These same concentrations have no effect on the chronaxie of the major responses.

Sensitivity to pitocin. The oxytocic principle is a true, probably physiological stimulant of the uterine muscle (13). Its uterine effects are due to its action upon the muscle alone (8); nervous elements are apparently not affected. Sensitivity to pitocin, therefore, indicates a muscular reaction.

The chronaxie of the minimal responses to electrical stimulation is affected by pitocin, as illustrated in figure 4. During proestrus and diestrus, concentrations of 1:500,000[4] pitocin decrease the chronaxie from its normal values of about 1600σ to low values of about 400σ. Higher concentrations have no further effect, and the low chronaxie values which are found normally during estrus and metestrus are not decreased any further by pitocin.

Comment. Judging by all the established criteria which we have been able to find, the minimal responses to electrical stimulation are reactions confined to the muscular elements of the uterus, in which nervous tissue is not involved. The chronaxie of the minimal responses therefore represents the electrical excitability of the muscle itself, as opposed to the chronaxie of the major responses which probably depends upon the neuromuscular complex

[3] In diestrous and proestrous uteri, the chronaxie of the minimal responses, which normally averages about 1600σ, is not further increased by quinine.

[4] 1 cc. pitocin (Parke, Davis Co.) (10 U) in 500,000 cc. water.

or may even be determined primarily by the excitability of the nervous elements. Since it is the chronaxie of the minimal responses which varies with the estrous cycle, it may be concluded that the hormonal influences, which we are about to consider, affect uterine activity by altering the physiological state of the muscular protoplasm of the uterus.

Hormonal Influences on the Chronaxie of the Minimal Responses

It has been shown previously (1) that the chronaxie of the minimal responses to electrical stimulation in the uteri of normal rats, decreases from an average value of 1600σ during proestrus to 400 to 500σ during estrus (fig. 4). A similar increase in excitability was observed during the estrus which followed the administration of estrogenic substances to normal rats. From these observations it appeared probable that the excitability of the uterine muscle as well as the histologic estrous phenomena were governed by the same estrogenic ovarian secretions. This assumption was not supported by the results of castration. Since it takes only 2 to 3 days for the chronaxie to rise from its lowest to its highest values during the estrous cycle (fig. 4) it was expected that a similar rapid change would follow the elimination of estrogenic influence by ovariectomy. Surprisingly enough, in spite of the anestrous vagina and the diestrous appearance of the uterus which appeared within 2 to 3 days after castration, low estrous-like chronaxie values were obtained for 2 weeks after the ovaries had been removed (fig. 5). During the third week the chronaxie increased to diestrous levels, but it is difficult to estimate how much of this change might have been due to the progressive uterine atrophy which was rendering our determinations difficult. After the third week the atrophy made further determinations impossible.

These results do not warrant the conclusion that the estrogenic hormones play no rôle in uterine excitability, for we have already mentioned the marked effect on chronaxie which may be obtained by administering these substances to normal animals. Furthermore, the continued administration of dihydroxyestrin benzoate[5] to our castrate rats, besides inducing an estrous uterus, also maintained low chronaxie values similar to those found in spontaneous estrous, for as long as 7 weeks after castration (fig. 5). It is clearly evident, however, that the rapid alternation between increased and decreased uterine excitability in the 5 to 6-day sex cycle of the intact rat, cannot be explained solely on the basis of an intermittent secretion of estrogenic hormone by the ovary. It seems necessary to postulate another factor, antagonistic to the estrogenic hormone, which appears as estrus ceases and rapidly decreases uterine excitability to its diestrous level.

In view of the foregoing, we administered a number of hormone preparations to our castrated animals to see whether uterine chronaxie would be affected. Although none that we tried raised the chronaxie values during the first two weeks after castration, APL substance[6] lowered the chronaxie

[5] Progynon-B (Schering Corp.) for supplies of which we are indebted to Dr. Gregory Stragnell.
[6] Antophysin (Winthrop Chemical Co.)

values to the estrous level, during the third week of castration (fig. 5). It may be that its activity resulted from other materials in the pregnancy urine extract. But if so, it was not due to estrogens as judged by histologic effects. Observations on the influence of APL substance could not be continued beyond the third week of castration because of the progressive uterine atrophy, which was as severe as in untreated, castrate animals.

There remained the possibility that the low chronaxie values after castration might be due to the well-known secondary effects of this operation on the other endocrine glands, notably the pituitary. Our results did not indicate this to be the case. One week after hypophysectomy, 6 rats (3 of them castrated) yielded low chronaxie values similar to those obtained after castration. Four adrenalectomized rats (2 of them castrated) gave the same results. It seems likely in view of the fact that hypophysectomy always and adrenalectomy usually interrupts the estrous cycle (14), that the effects of the removal of these glands on uterine excitability depend, like castration, upon cessation of ovarian function.

Comment. It has been shown that the estrogenic ovarian hormones and the APL substance of pregnancy urine, in vivo, and the oxytocic principle of the posterior pituitary gland, in vitro, are all capable of inducing the same degree of excitability of the uterine muscular protoplasm as occurs in spontaneous estrus. But, in view of the prolonged period of similar excitability which follows castration, hypophysectomy, and adrenalectomy, none of the above effects can explain the rapid decrease in uterine excitability which occurs between the estrous and diestrous phases of the normal estrous cycle. It has therefore been necessary to postulate an as yet unidentified factor or hormone in the ovary, which actively decreases uterine excitability at the appropriate time in the cycle. This inhibitory factor is probably not derived from the corpus luteum since the excitability of the uterine muscle is greatest during metestrus and in pregnancy, when the corpus luteum is prominant. Harne (4) was forced to a somewhat similar conclusion in explaining his observations on uterine motility. This conclusion is also in agreement with the previous work of one of us (S.S.) and collaborators, which indicated the existence of an ovarian factor in the rat, which inhibits the histologic uterine response to estrin, and which operates in a cyclic manner corresponding to the estrous cycle of the animal (15, 16). It is interesting to speculate upon the potential importance of an analagous mechanism in the etiology and treatment of clinical disorders of sex function, if and when a similar inhibitory factor is identified in the human being.

SUMMARY

The spontaneous contractions of the rat's uterus, and the uterine responses to electrical stimulation were studied with a lever system which made it possible to record simultaneously in an intact uterine horn, the respective contractions of the vaginal end, of the ovarian end, and of the entire organ. It was found that the minimal responses to electrical stimulation were not analagous to any spontaneous uterine contractions. Judging by all the

available, established criteria, these minimal responses were reactions confined to the muscular elements of the uterus, without involving the nervous tissues. The chronaxie of the minimal responses had been shown previously to vary with the estrous cycle, and was used in the present work to study the effect of various hormones on the excitability of the muscular protoplasm of the rat's uterus. It was found that estrogenic hormone, APL substance and pitocin increased the excitability of uterine muscle to levels comparable to those which occur in spontaneous uterine estrus. Nevertheless castration, hypophysectomy and adrenalectomy, were followed by a similar increase in uterine excitability which lasted for two weeks. It was concluded that the ovary probably elaborates a factor or hormone which actively decreases the excitability of the uterine muscle at appropriate times in the sex cycle. The corpus luteum hormone is apparently not the substance involved. Such an inhibitory factor might have potential importance in the etiology and treatment of clinical disorders of sex function.

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GONADOTROPIC HORMONE: CLINICAL APPLICATION OF EXTRACTION METHODS FOR ASSAY PURPOSES[1]

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THE TWO MAIN obstacles which must be overcome to make possible an accurate assay of non-pregnancy urinary gonadotropic hormones are: *a*), a reliable assay end-point, and *b*), a satisfactory concentration method. The first obstacle has been satisfactorily removed (1) with the thorough investigation of the immature rat uterus and ovary as end-points for the assay of gonadotropic hormones. Overcoming the second is of primary concern at this time.

The reports of efforts in this direction are legion, indicating the general dissatisfaction with the concentration methods now extant. For the routine assay of large numbers of urine specimens from many different patients, any urine concentration method must meet the following prerequisites: *a*), It must be simple, in order that large quantities of individual specimens may be handled. *b*), It must concentrate the active fraction many times; because of the low content of some urines, the gonadotropic activity of from 500 cc. to 1000 cc. of urine must be concentrated into an amount small enough to inject into one rat. *c*), It must retain a large percentage of the original activity. *d*), It must give consistent and regular results for the sake of comparison between individual specimens. And *e*), it must meet all the above requirements without being toxic to the test animal.

Without reviewing each method encountered in the literature, it can be seen that the above requirements will a priori eliminate the majority of methods now in use, or previously proposed. Most of the methods have been for intensive study of the product of the concentration procedure, and not developed as daily routine clinical procedures for determinations on individual specimens. Investigations in this laboratory (by Dr. Leita Davy) have indicated that concentration methods developed for pregnancy urine are not usually applicable to normal and menopausal urines. Therefore pregnancy urine concentration methods have not been attempted.

Of all the procedures for gonadotropic concentration of non-pregnant urine attempted, only those few which showed any promise of meeting our requirements will be mentioned, namely the alcohol-ether precipitation method originally used by Zondek (2), and later used by Damm (3), Albright, Halsted and Cloney (4), Saethre (5), Büttner (6), Kräne (7), Jones and Mac-

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Gregor (8), and others; the tannic acid method of Levin and Tyndale (9), and Thomsen and Pedersen-Bjergaard (10), and the modifications introduced by Sulkowitch (11), D'Amour (12), and Levin (13), as well as a saturated ammonium sulfate method devised in this laboratory. This last was discarded after considerable experimenting due to its complexity, lack of completeness of recovery, and its toxicity.

For the purpose of *a*), comparing the alcohol-ether and tannic acid methods, and modifications thereof, *b*), in order to test the extent of possible recovery, and *c*), to determine the consistency of the methods, 37 l. of urine from several menopausal patients were collected and pooled. From this pooled sample 500 cc. aliquots were removed and concentrated for injection. The test animals used were all 21-day-old female rats from the Sprague-Dawley colony. All urinary precipitates were dissolved in that amount of water necessary to administer a 0.5 cc. to 1.0 cc. injection twice daily for 3 days. Autopsies were performed on the 4th day. Uterine and ovarian weights, and vaginal opening were used as criteria of gonadotropic stimulation.

Numerous 500 cc. aliquot samples were extracted separately at different times, and 3 or more rats were injected with the same dose from the same aliquot, in order to test for consistency of recovery and response. After preliminary trials a 150 cc. equivalent of the pooled urine was decided upon as the optimal dose per rat, and this dosage was used in comparing the methods, the regularity, and amount of recovery. The methods tested were run simultaneously in order to insure comparable responses.

The tannic acid method of Thomsen Pedersen-Bjergaard was first tested. The concentrate obtained by this method proved to be too toxic, and a new step suggested by Dr. Mark Foster was introduced. After the acetone-washed tannate precipitate had been collected at the centrifuge, 40 to 60 cc. of 50 per cent aqueous pyridine was added, left to stand overnight, and precipitated with 4 volumes of 95 per cent alcohol, and the centrifugate taken up in water. A comparison of the methods can be seen in table 1. The Thomsen Pedersen-Bjergaard method urines are designated as T. A.; the pyridine treated tannates as P.T.A. The injections were made subcutaneously in the back. Swelling and soreness resulted, and hematomas formed with all these concentrates. The ovaries all showed negative findings, while the uteri were quite variable in their response. Therefore, it was concluded that the pyridine method as we employed it was unsatisfactory; as was also the tannate method.

Using the same pool and the same scheme, 500 cc. aliquots were precipitated with 4 volumes of 95 per cent ethyl alcohol, left to stand overnight in the refrigerator, and the alcohol-urine supernatant liquid syphoned off. The residue was centrifuged, and the precipitate was washed once with ether and dried in front of a fan. The dry powder was eluted 3 times with tap water, the suspension being centrifuged each time and the supernatant liquid saved. The results are averaged and compared with the above two methods in table 1.

TABLE 1. COMPARISON OF THE TANNIC ACID, PYRIDINE TANNIC ACID AND ALCOHOL-ETHER CONCENTRATION METHODS ON THE SAME 37-LITER POOLED MENOPAUSE URINE SAMPLE

Method	cc. equivalents	Average ut. wt. mg.	Average ov. wt. mg.	Toxicity	No. of rats
T. A.	150 cc.	68	12.7	Toxic	14
P. T. A.	150 cc.	35	11.3	Less toxic	33
EtOH	150 cc.	119	42.1	Not toxic	20
Normal control	Uninjected	19.6	12.4		55

The table shows the average response of the number of rats used at the 150 cc. equivalent dose level for each method.

The alcohol-ether method was varied to see whether recovery could be still further improved. For example, the samples were precipitated with 4 volumes of alcohol at varying H-ion concentrations. The urine was adjusted to pH 4.7 and pH 6 by adding acetic acid, and to pH 10 with ammonium hydroxide. No essential difference was found between urines having a pH of 4.7 or a pH of 6.0, but those at pH 10 precipitated poorly and were toxic.

Another modification was tried. The ether-washed precipitate was eluted with borate buffer at pH 9.5 instead of tap water, and the final supernatant liquor readjusted to pH 7.0 with acetic acid. No differences were noted, so it was decided as our regular procedure to precipitate the urine without pH adjustment (unless a specimen is definitely alkaline), and to elute with tap water.

Table 2 shows the consistency of the results obtained with the alcohol-ether method when 500 cc. aliquots were precipitated and prepared separately over a period of several weeks. The uterine weight and ovarian weight figures represent the average of the number of animals on each dose level of each separate sample. Doses of 150, 75, 50, and 25 cc. equivalents were used and compared with one another. Closer inspection of table 2 reveals that the

TABLE 2. ALCOHOL-ETHER CONCENTRATE: SHOWING THE CONSISTENCY OF RESPONSE AT VARIOUS DOSE LEVELS, WHEN SEVERAL 500 CC. ALIQUOT SAMPLES OF THE SAME 37-LITER POOL ARE COMPARED

No. of rats on 150 cc. dose	No. of rats on each other dose	Ovary weights mg.					Uterus weights mg.				
cc. equivalents		150	75	50	25	Uninjected control	150	75	50	25	Uninjected control
	55					19.6					12.4
3	0	36.5					132				
3	0	45.5					122				
3	0	44.3					124				
3	0	48.6					113				
2	4	40.9	23.2	14.8	12.9		121	117	114	46	
1	2	50.2	20.6	14.4	11.4		114	109	111	43	
1	2	42.8	19.3	13.1	13.2		119	101	115	102	
1	2	24.0	20.8	13.1	12.9		111	96	101	73	
1	2	49.3	17.1	14.2	13.3		114	116	132	110	
1	2	28.4	26.2	12.8	12.5		103	98	124	64	
1	2	40.6	18.1	14.2	10.5		107	112	126	49	

ratio between minimal uterine and minimal ovarian response will not be 1 to 6 or 8 as previously reported for rat pituitaries (1), but will be closer to a ratio of 1 to 4. Subsequently, it has been found that the ratio for urines of individual patients is about 1 to 2 or even 1 to 1. Therefore, the ovaries take on greater importance as an assay criterion than they did in assaying pituitaries from castrate animals treated with estrin (14).

It was found that urine preserved with boric acid and kept at 36°C. did not begin to show signs of losing potency until 3 months from the time of collection. The ether-dried precipitate can also be kept in the ice box for months without any appreciable loss in potency. Eluted samples show no

TABLE 3. TESTS ON THE URINE OF SEVERAL PATIENTS FOR AMOUNT OF RECOVERY OF THE ALCOHOL-ETHER METHOD WHEN COMPARED WITH THE ORIGINAL UNCONCENTRATED URINE. EACH RAT RECEIVED 10 CC. OVER A 5-DAY PERIOD

Patient	Unconcentrated			Alcohol-ether concentrate		
	Vaginal opening	Uterine weight − fluid mg.	Ovarian weight mg.	Vaginal opening	Uterine weight − fluid mg.	Ovarian weight mg.
J.	−	28	11	−	29	16
	−	34	16	−	30	15
	−	26	16	−	30	12
S.	−	52	12	−	28	19
	−	38	12	−	28	15
	−	25	12	−	27	13
Sh.	+	94	40	−	26	13
	+	112	26	−	26	20
	−	46	10	−	22	17
K.	−	42	12	−	32	15
	+	164	34	−	40	20
Kr.	−	52	24	+	80	53
	−	23	8	+	66	95

loss in 2 weeks time, but by 3 weeks, the activity is materially decreased. It was discovered that if the urine was warmed at room temperature an hour before precipitating, the precipitate came down with greater ease and was subsequently easier to handle. No toxic effects were noted in our regular dose levels of injections. However, at higher levels of injection, soreness and hematomas frequently developed at the site of injection. While this is not desirable, it does not interfere with the results of the tests, unless leakage occurs. However, by reprecipitating the final solution with 4 volumes of alcohol, the toxicity can be remarkably reduced without losing appreciable activity.

In order to test the amount of recovery of the alcohol-ether method, individual specimens of raw urine from several patients were shaken with ether (the residual ether was evaporated off with a stream of air) and injected into 24-day-old rats in 10 cc. amounts (1cc. injected twice daily for 5

days). The rats were killed on the 6th day. Ten cc. equivalents of the alcohol-ether concentrate from the same specimens were also injected for identical periods. Table 3 shows the comparisons. In some instances the raw urine and the alcohol-ether concentrate elicited the same results. In other cases either the concentrate or the raw urine gave a greater response.

Thus the advantages of the alcohol method are: *a*), It is simple and direct, that is, it is being performed by a non-technical worker with good, consistent results in routine testing. *b*), It is convenient, the products can be stored at any stage. *c*), It concentrates the active gonadotropic fraction many times. *d*), It recovers a large percentage of the potency (the test for recovery was done along with routine clinical specimens without the worker's knowledge that a test of his technic was being made with raw urine, at the same time). *e*), Variation in amount of potency recovered and responses of animals is minimized. *f*), It is less toxic than any other method attempted in the lower doses.

Acting upon the suggestion of Levin (13) that to reduce the toxicity of the tannic acid method, fresh urines be immediately precipitated with reagent grade tannic acid, adding 40 cc. per liter of urine, and by carefully following the Levin and Tyndale technic, some reduction in toxicity was obtained. Thus 500 cc. and 600 cc. equivalents could be successfully injected into one rat. Again the backs showed signs of injury, but no other toxic effects were noted. In our hands however, this method offers slight advantage over the alcohol-ether method, since on actual routine assays of individually run urines usually the same response produced by tannic acid at 500 cc. equivalents can also be produced by the alcohol-ether concentrates at the 200 cc. equivalent level.

Numerous comparisons between the two methods, and also subsequent experiments varying the technic of the tannate method according to suggestions by D'Amour and Sulkowitch did not result in any material improvement. Levin recommends eluting the final precipitate while D'Amour recommends suspending the entire precipitate.[2] The two were compared on several identical samples. In no instance was eluting as effective as suspending, although condition of the animals was better in those receiving the eluted solutions. The discards from samples eluted at pH 9.5 were tested in some cases and these were found to have retained some potency. Therefore, elution with borate buffer really adds a step which reduces the completeness of recovery.

In order to determine the completeness of recovery from routine assays of urine from various individuals, we had previously checked the response elicited by the alcohol-ether method, with raw urine. This experiment was repeated using a larger number of rats and a pooled sample of urine from a single patient whose gonadotropic excretion rate was known to be high. Three different methods of concentration were employed. The tannic acid

[2] We wish to express our appreciation to Dr. Louis Levin of Columbia University College of Physicians and Surgeons, Dr. Hirsh Sulkowitch of the Massachusetts General Hospital, and Dr. Fred E. D'Amour of the University of Denver, for their kind suggestions.

method was very carefully carried out by one of us, following the Levin and Tyndale technic exactly. A similar aliquot of the pool was concentrated using the alcohol-ether method and a third aliquot of raw urine, after being shaken with ether, was then injected per se. Results of the comparisons may be seen in table 4.

The 25 cc. equivalent doses were injected over a 3-day period, the 8 cc. dose over a 4-day period, and the 10 cc. amounts over a 5-day period. Rats injected with the 8 and 10 cc. equivalents were litter mates, and all technic

TABLE 4. COMPARISON OF THE ALCOHOL-ETHER AND TANNIC ACID METHODS WITH UNCONCENTRATED URINE

Litter No.	Dose cc.	Unconcentrated				Tannic acid				Alcohol-ether			
		Vaginal opening	Ut. wt.+ fluid mg.	Ut. wt. − fluid mg.	Ovarian weight mg.	Vaginal opening	Ut. wt.+ fluid mg.	Ut. wt.− fluid mg.	Ovarian weight mg.	Vaginal opening	Ut. wt.+ fluid mg.	Ut. wt.− fluid mg.	Ovarian weight mg.
	25					+	174	84	38.0	+	250	100	57.4
	25					+	188	83	45.6	+	196	98	48.9
	25					+	209	84	39.8	+	219	90	60.0
Average		Ovary weight							41.1				55.6
a.	8	+	240	128	13.6	−	—	35	14.2	−	68	59	20.4
b.	10	+	152	140	23.0	−		31	17.0	−	236	112	21.0
c.	10	+	91	86	45.8	−		38	13.6	+	168	104	18.2
d.	10	+	272	141	16.0	−		28	13.6	−	143	82	17.0
e.	10	+	79	76	49.6	−		44	16.2	+	104	100	33.8
f.	10	+	116	115	39.8	−		52	13.4	+	96	94	44.2
g.	10	+	134	130	59.2	−		32	20.2	+	307	132	28.0
h.	10	+	145	140	42.6	−		22	15.0	+	175	105	20.2
i.	10	+	130	128	28.0	−		28	13.4	+	230	121	25.8
		Aver. ovary weight 38.0							15.3				26.0

The rats receiving the 25 cc. dose were injected for 3 days, the 8 cc. dose for 4 days, and the 10 cc. dose for 5 days.

was done as uniformly as possible. From the table, it is seen that, of the two concentration procedures, the alcohol-ether method gives the greater amount of recovery, although the raw urine control shows that this cannot be considered to be 100 per cent.

SUMMARY

Several concentration methods of urinary gonadotropic hormone for assay purposes were compared with untreated urine to test their completeness of recovery. Several consecutive tests were made from the same pooled specimen to test the regularity of results and the methods were varied until the simplest satisfactory procedure resulted. The alcohol-ether concentration method essentially fulfills all requirements of an adequate extraction method for clinical use, and has proved its worth in routine assays in our laboratory.

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ADDENDUM

Since this paper was submitted, we have studied the excretion of gonadotropic hormone by normal and menopausal women (15), and by normal males. When high doseage levels of extracts from normal young males and females were injected, toxicity was encountered. The animals often died after 1 or 2 injections. This toxic reaction seemed to be limited to the urines of young adults, and was not encountered in working with menopausal urines. It was suggested by Dr. E. L. Sevringhaus that salts, probably chiefly sodium chloride, might be taken up in the extracts and cause the toxicity. After the salts were removed from the final eluted solutions by dialysis against tap water in collodion bags, solutions previously so toxic that the rats died, became non-toxic. The condition of the site of injection was also greatly improved. The increased volume of the solutions led us to evaporate it to the original 6 cc. level by a stream of compressed air. The potency of the extracts was not reduced by dialysing as long as 24 hours. Evaporation did not affect the solutions provided they were not decreased below the 6 cc. level. Evaporation to dryness caused a loss in potency. Enough of the salts are removed by dialysis for 2 or 3 hours to render the solution non-toxic. With this method, we are now able to inject the equivalent of 1000 cc. of urine from young adults into one rat without toxicity. Dialysis is now included as a routine part of the procedure of extraction for all solutions in which 400 to 1000 cc. equivalents of urine are to be injected.

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STABILITY OF TESTIS HORMONE

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OF THE NUMEROUS artificial and natural androgens known, one of the most active is testosterone which was isolated from the testes by Laqueur (1) and his associates. With the exception of the reported isolation of androstanedione by Ogata and Hirano (2) no other hormone has been prepared from testicular tissue but there are indications that testosterone is not the only internal secretion of significance produced by the testes. The question of the duality or possibly the plurality of testes hormones has been studied in this laboratory for some time. The experiments concerning the relative stability of testosterone and testicular extracts which are reported in this paper throw additional light on this subject.

METHODS AND RESULTS

The effect of boiling testosterone with acid. Three hundred micrograms of testosterone were dissolved in 750 cc. of ethyl alcohol which was mixed with 150 cc. of concentrated sulphuric acid and sufficient water to make the final volume 3l. This solution was divided into 3 equal parts which were treated in the following manner.

a), One liter was extracted thoroughly with dibutyl ether. The extract was washed with dilute sodium hydroxide and water and evaporated to dryness using steam distillation and a partial vacuum. The residue was dissolved in 10 cc. of sesame oil and assayed using 10 capons. The oil was inuncted onto the combs of the capons over a period of 5 days. Each bird received one-fifth of 1 cc. daily, this being spread evenly over the whole comb. One day after the final inunction the combs were measured. Growth is expressed as mm. increase in length plus height.

b), The second liter was boiled under reflux for 15 minutes and then treated as was sample *a*.

c), The third sample was boiled under reflux for 5 hours and extracted and assayed as was *a*.

The average growths caused by preparations *a*, *b* and *c* were 7.1, 6.5 and 4.9 mm. respectively. It is apparent from these results that, under these conditions testosterone is very slowly inactivated by boiling with 5 per cent sulphuric acid by volume. Even after boiling for 5 hours the testosterone retained about 70 per cent of its activity.

The effect of boiling testis tissue extracts with acid. Six hundred cc. of testis tissue was ground to a fine mush and rapidly heated to 90° C. by passing superheated steam into the vessel. This caused a coagulation of proteins from which a considerable aqueous expressate could be drawn. The active androgens were extracted from this material with dibutyl ether, in which

all the known active androgens are very soluble. The ether was removed by steam distillation under partial vacuum and the residue taken up in 50 per cent alcohol. The alcoholic solution of hormone was divided into two equal portions. One was reextracted with dibutyl ether, taken to dryness, taken up in oil, assayed by the inunction method using 5 capons, and found to cause a growth of 5.8 mm. The other sample was boiled for 15 minutes with 5 per cent sulphuric acid by volume prior to being assayed in a similar fashion. It caused a comb growth of only 2.8 mm. As demonstrated above, testosterone is only very slowly destroyed by boiling with acid. The rapid decrease in androgenic potency of the testis tissue extracts when boiled with acid indicates that the androgenic activity is not entirely due to testosterone. The activity of the androgens of testes was decreased about 50 per cent in 15 minutes while the activity of testosterone was decreased only about 10 per cent in 15 minutes.

It has recently been shown that all the androgenic material of human male blood and urine is in an inactive form which is insoluble in dibutyl ether but soluble in alcohol (3). Therefore, another series of extracts was prepared containing both active and inactive androgens. Bovine testes were frozen at the abbatoir immediately after the death of the animals. These were finely ground at the laboratory and 1210 cc. of the ground tissue were added to 1300 cc. of 95 per cent ethyl alcohol. The mixture was filtered through cotton gauze, the filtrate diluted with an equal volume of water, and divided into 3 equal portions. The first portion was extracted with dibutyl ether and the extract assayed in the usual fashion. It caused an average comb growth of 8.7 mm. using 10 birds for the assay. The second portion was boiled for 15 minutes with 5 per cent sulphuric acid before being extracted and assayed. This extract was more active as a result of the boiling and caused an average growth of 10.0 mm. in each of 10 birds. Following boiling of the third portion in the same fashion for 5 hours, the resultant comb growth on assay was only an average of 7.7 mm. in each of 10 birds. Thus we see that testes, like blood and urine, contain a substance the androgenic potency of which is increased by boiling with 5 per cent sulphuric acid for a short period and that some of the activity is lost by prolonged boiling.

DISCUSSION

These simple experiments using only 70 capons prove the existence of more than one androgen in the testes. They are supported by other preliminary unpublished experiments involving the use of about 200 capons. Whether or not more than one androgen is secreted into the blood stream cannot be said. This proof of the existence of more than one hormone in the testes helps to explain some otherwise puzzling facts and makes it possible to suggest some interesting hypotheses which may act as useful guides in further studies of testicular physiology. Since the literature concerning the recent advances in this field has been excellently reviewed by Koch (4) and by Wintersteiner and Smith (5), it seems necessary to mention here only very recent papers and observations pertinent to this discussion.

It has been established that the feeding of desiccated testicular material to patients suffering from benign prostatic hypertrophy will in many instances relieve them of their symptoms (6). Van Cappellen (7) has shown that the injection of testosterone will also relieve the symptoms of that disease. In that testosterone is poorly absorbed when administered orally and in that there are very small quantities of testosterone present in the testes, it seemed improbable that the results obtained by feeding desiccated glandular material were due to testosterone. Formerly the effect has been credited to the existence of a hypothetical hormone 'inhibin.' It is not impossible that the clinical results observed are due to the existence in testicular material of an androgen or related compound other than testosterone. One observation indicates that possibly the inactive androgen in testes might be the substance effective in the treatment of prostatic hypertrophy. As has been demonstrated above, the inactive androgen of testes is activated by boiling with acid. In unpublished experiments in this laboratory, it has been shown that the inactive androgens of blood and urine can be activated by tissue extracts. This is no doubt an enzymic activation quite comparable to the hydrolysis of estriolglucuronide (8). It has been noticed that testis tissue desiccated over a period of 2 or 3 days at about 30° C. is not as effective in the treatment of prostatic hypertrophy as is material prepared at a temperature of 65° C. The higher temperatures inactivate enzymes present in the tissue and thus may prevent changes in the testicular hormones making them ineffective in the treatment of prostatic hypertrophy.

Moore and Price (9) have studied the effect of various doses of testosterone on the prostate and seminal vesicles of castrated rats and find that it is possible to give a dose which will stimulate these organs in such a fashion that they will retain their normal weight relationships. From this they conclude that testosterone does not exhibit a more selective stimulation on any one end organ than does normally secreted testis hormone. Cutuly, McCullagh and Cutuly (10) find that in hypophysectomized rats the relative potency of the various androgens depends on the end organ being considered. Thus, of nine androgens investigated, testosterone propionate was definitely superior to the others in maintaining the weight of the secondary sex glands, but was fourth in its effectiveness in the maintenance of testicular weight. There are no two known androgens, artificial or synthetic, which have identical pharmacological properties when considered quantitatively. It is improbable that testosterone alone can completely replace the testes with respect to their endocrinological function.

The relationship of the androgens to spermatogenesis can best be explained by assuming the normal existence of androgens other than testosterone. It has been demonstrated that androgens will maintain spermatogenesis in hypophysectomized rats and will stimulate the testes of immature hypophysectomized rats. These effects are very marked and it seems probable that under normal conditions one function of the internal secretion of the testes is to play a rôle in the support of the whole of the fecundatory mechanism including spermatogenesis. Moore and Price (9) report an ap-

parently paradoxical reaction of the testes of the normal rat to testosterone. They find that this androgen is injurious to the testes of the normal rat and that over a certain range the suppression of testicular growth increases as the dose is diminished. This result appears paradoxical only if one considers testosterone to represent the only androgen produced by the testes. The following explanation, although not proved, seems very plausible.

Existing theories concerning testis-pituitary interrelationship would indicate that if one administered an androgen qualitatively physiologically equivalent to the testis hormone (or perhaps combined androgenic hormones of testes) there would probably be no disturbance in the amount of androgen available to the animal unless very large doses were used. For example, if one administered an amount of hormone equal to one-half of that which the animal normally produces, it would seem possible that the pituitary gland would be sufficiently depressed that the testes would decrease in activity to such an extent that the normal amount of androgenic material would be present. According to this point of view, it would seem possible that testosterone is not the physiological equivalent of testicular androgenic material of the testes. In large doses it depresses the pituitary gland and maintains the testes. Doses insufficient to maintain the testes depress the pituitary so greatly that the testicular hormone (or androgenic complex) is no longer produced in sufficient quantities to maintain spermatogenesis satisfactorily.

To those who have supported the idea that there exists more than one active principle in the testes, it has seemed very important that aqueous extracts of testicular material showed pharmacological activity whereas the known androgens are very soluble in the fat solvents and almost insoluble in water. This fact is really of minor significance. It is impossible at present to know what substances other than androgens and other recognized tissue constituents are present in extracts of testes. How these substances might affect the solubility of the androgens is equally unknown. For example, Windaus (11) has shown that in the presence of the sodium salt of cholestenone-6-sulphonic acid, the solubility of sterols in water is quite high. In this laboratory it has been observed that most of the ether-soluble androgenic material of bovine testes is present in the aqueous expressate which can be obtained after coagulating the proteins of the ground testes with heat. The discovery of the existence of more than one androgen in the testes makes it necessary to consider the possibility that part of the androgenic complex of the testes may be truly water-soluble. Whether or not this is so can be ascertained only after isolating the hormone.

In the early years of this work, the fact that the properties of water-soluble extracts and the androgens should be compared was not overlooked. Walsh, Cuyler, and McCullagh (12) demonstrated by means of the parabiotic technique that both androgens prepared from human male urine and aqueous extracts of testis tissue would prevent the hyperactivity of the pituitary gland of castrated rats. At that time, no satisfactory explanation for the findings was offered. The fact that active androgens other than testosterone exist in testicular material offers a possible explanation.

The influence of the gonads on the metabolism of creatine and creatinine has also entered the picture of the duality of hormonal production by the male gonads. In a series of papers by Zwarenstein (13) and others they showed that, although the effects are opposite, both saline suspensions of testes and crystalline androgens influence creatinine metabolism. They suggest that this indicated the existence of two testicular principles. There is nothing to indicate whether or not they consider the two hormones to be chemically related.

In considering these facts, it becomes obvious that at present it is impossible to construct anything approaching a complete picture of the physiology and biochemistry of the testes. It is equally certain that in attempting to do so it must be remembered that extracts of testes with varying biochemical and pharamacological properties can be prepared and that there exists more than one endocrinologically active principle in that gland.

SUMMARY

Testosterone is more stable in boiling 5 per cent sulphuric acid than are testicular extracts. The androgenic activity of bovine testes is increased by boiling with acid for 15 minutes. The influence of these findings on concepts of testicular physiology is discussed.

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THE EXCRETION AND FATE OF ANDROGENS[1]

THE EXCRETION OF INJECTED TESTOSTERONE AND ITS VARIOUS DERIVATIVES

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A PRECEDING paper described (1) the fate in the animal body of urinary androgen extracts and a synthetic androgen, androstenedione. The results obtained with both products were similar; only 2 to 6 per cent of the activity of the injected hormone could be recovered in the urine.

On the basis of the results obtained with androstenedione, it was expected, because of the similarity in chemical structure, that an equivalent loss in activity would occur with testosterone. It was demonstrated that the free androgens whether saturated or unsaturated were inactivated in the body (1). The experiments to be reported here were designed to determine whether protection of the polar groups would either prevent or retard this inactivation.

EXPERIMENTAL METHODS

Materials. The various androgens[2] were synthetically prepared crystalline substances and were dissolved in either olive or sesame oil.

Procedure. The animals, 4 adult male castrate dogs, were kept in metabolism cages. The androgens were injected either in a single dose or in 2 to 3 successive daily doses. The urine was collected daily under toluene.

Extraction of Urine. The combined urine for each period was extracted as previously described (1, 2).

Assay. White Leghorn capons were used for assay by the 5-day method of Gallagher and Koch (3). This method requires a minimum of 500 γ androsterone (or its biological equivalent) to produce a measurable response in the capon's comb. The small amount of androgen excreted necessarily limited the number of animals used for each assay. The total extract of each period was injected into two birds in every case except the first period in experiments 3 and 7 where three were used.

RESULTS

The results are compiled in table 1. The activity of the respective derivatives of testosterone are presented in terms of their testosterone content. The activity of each compound by itself, however, varies markedly. The benzoate is not assayable by the capon's comb method. The oxime has approximately $\frac{1}{3}$ the activity of testosterone and the acetate and propionate have a slightly greater maximum effect which is distinctly prolonged.

[1] This investigation was supported by a grant from the International Cancer Research Foundation.
[2] These substances were provided through the courtesy of D. E. Schwenk of Schering Corporation and Dr. E. Oppenheimer of Ciba Pharmaceutical Products.

The most striking result is the very high loss in activity which is even greater than in the case of androstenedione and urinary androgenic extracts (1). This, however, may be readily explained by the fact that testosterone is approximately 5 times as active per unit weight as androstenedione and the most active constituent of the urine extract, androsterone.

Of the 7 experiments only 2 yielded sufficient active material in the urine to allow an assay. Furthermore, when less than approximately 120 mg. of hormone was injected an assayable quantity was not obtained in the urine.

TABLE 1. EXCRETION OF INJECTED ANDROGENS

Expt.	Androgen	Dosage			Period	Days	I.U. Excreted	Percentage recovered
		mg.	as testosterone[1] *mg.*	I.U.				
A.	Testosterone	3×15		2250	1	6	0[2]	0
					2	7	0	0
					3	10	0	0
B.	Testosterone	3×20		3000	1	6	0	0
					2	7	0	0
					3	11	0	0
C.	T. Oxime	2×64	121.7	6085	1	6	15	0.25
					2	5	0	0
					3	5	0	0
D.	T. Benzoate	2×25	36.8	1840	1	6	0	0
					2	5	0	0
					3	5	0	0
E.	T. Acetate	3×20	52.4	2620	1	8	0	0
					2	7	0	0
F.	T. Acetate	3×25	65.5	3275	1	8	0	0
					2	8	0	0
G.	T. Propionate	1×238	199.2	9960	1	2	35	0.35
					2	2	0	0
					3	2	0	0
					4	2	0	0
					5	2	0	0

[1] 1 mg. = 50 I.U.
[2] Cf. under assay.

Protection of the polar groups of the testosterone molecule apparently does not alter its fate. In experiments *A* and *B* in which 45 and 60 mg. of testosterone were used no detectable active material was found in the urine. Protection of the keto group by oximation resulted in a 0.3 per cent excretion, but this was due to the higher dosage rather than the protective effect of oximation. The 17 hydroxyl group was protected by production of 3 different esters. In no instance is there an appreciable excretion of androgenic material. The benzoate presents an interesting case. It was expected that injection of this compound would result in the appearance of large amounts of androgenic material in the urine due to the protective action of the acid group. Instead it falls in line with the other compounds. It would seem, therefore, that although benzoylation does not allow the molecule to perform its

physiological action (4, 5, 6) the substance, nevertheless, is still susceptible of inactivation.

The active material excreted appears within 2 days after injection and subsequent days yield no further activity. This is even more striking when we consider that such results were obtained with testosterone propionate. This compound is known to have a greater effect than testosterone on the accessory sex glands of castrate white rats (6, 7, 8). The greater effect is believed to be due to a retardation in the rate of absorption (9). On the other hand, testosterone acetate, which behaves like the propionate produces neither a greater nor a more prolonged effect on protein retention in castrate dogs than does the free testosterone (10).

DISCUSSION

The high loss in activity of injected testosterone and its various derivatives indicates that the body possesses some mechanism for inactivating the androgens. It is significant that the percentage excreted is about the same regardless of the amount injected. It seems probable that the hormones take part in the reaction, rather than acting as catalysts and that during this process the molecule undergoes changes. If such is the case then our conception of androgens as catalysts must be revised. Furthermore, since the androgens normally excreted in the urine are also inactivated by the body after injection (1), it seems that these substances are not the ultimate end products of the original or testis-produced compound or compounds. This becomes more apparent when we consider that there are several products excreted in normal urine. These compounds have only slight variations in chemical structure which, however, notably affect their physiological activity. The relative potency of these compounds on the capon's comb is as follows: androsterone 1 (11), dehydroandrosterone 1/3 (12), isoandrosterone 1/7 (13) and epietiocholandiol 0 (14). The most active of these, androsterone, has only one-fifth the potency of testosterone. Although the activity of these compounds is less than that of testosterone and they appear in the urine, it seems premature to call these substances waste products. It seems more fitting that they be classified as intermediates produced to better bring about a specific physiological effect. For example, urinary androgenic extracts have a greater effect than testosterone in regenerating the prostate of castrate white rats (10).

The small amount of androgenic substances excreted after the injection of androgens makes it difficult to determine the exact chemical nature of these substances. Preliminary investigations in this laboratory indicate that the substances excreted, after the injection of androstenedione, do not possess the α, β unsaturated grouping in the molecule. Steinach and co-workers found substances in the urine with estrogenic activity after the injection of urine extracts and androsterone (15) and testosterone propionate and androsterone benzoate (16). These workers, however, neglected to separate the neutral fraction of the extracts from the phenolic fraction. Consequently, the estrogenic activity may have been due to either true estrogens or andro-

gens with estrogenic activity. Apparently they were assaying the latter for it has been demonstrated in this laboratory that no true (phenolic) estrogens appear in the urine (2) after the injection of various androgens.

Callow (13) has studied the recovery of androgens after the administration to human beings and found a loss in activity of the same order reported in our previous (1) and present papers. The uniformly similar loss in activity of injected androgens would suggest that we might consider the amount appearing in the urine to be a criterion of that produced in the testes. It is difficult, however, with our present knowledge to place a definite physiological reason for the elimination of these compounds. We know that they appear in the urine in a conjugated form similar to toxic compounds, but it does not seem to be compatible with vital economy to consider these compounds as toxic substances.

SUMMARY

After the subcutaneous injection of oil solutions of testosterone and its various derivatives—acetate, propionate, benzoate and oxime—into 4 castrate dogs, there was recovered from the urine about 0.3 per cent of the original activity (capon units). Protection of the polar groupings did not prevent the inactivation of the molecule or delay or prolong the rate of excretion. The assayable material was found in the urine of the first 2 days after injection.

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THE DAILY EXCRETION OF URINARY ANDROGENS IN NORMAL CHILDREN[1]

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FROM STUDIES in this laboratory, as yet unpublished (1), it has been determined that the 24-hour urinary excretion of estrogens in both the male and female child vary from day to day. In young children (5 to 10 years of age) these fluctuations are slight, but in the pre-adolescent groups they become more marked. Our data obtained thus far suggest that in the female child, at least, there is a cyclic excretion of the estrogenic hormones for several years before the menarche. This necessitates, therefore, the assay of a series rather than a single day's specimen of urine in the study of the normal estrin excretion even in children. The present study was undertaken to ascertain if there were similar variations in the daily output of the urinary androgens before puberty.

Androgens in the urine of children have been reported by Kochakian (2), Pederson-Bjergaard (3) and others. Recently, single determinations of groups of children have been made (4, 5, 6, 7). The latter two groups of investigators utilized a colorimetric method based on Zimmerman's (8) reaction for the detection and assay of these hormones. Wu and Chou (9) did not correlate their results with capon assay, although this was carried out by Oesting and Webster with rather satisfactory results. In the present work we have employed the colorimetric method of Oesting (10) for the titration of the urinary androgens. Although it is a non-specific reaction, we have found it to be very satisfactory, because of its simplicity. The only variation from the technic described by Oesting is in the method of hydrolysis. Since our work on the estrogens has been carried out on urine hydrolyzed with hydrochloric acid and extracted with benzol according to the method of Smith and Smith (11), we have continued to utilize this procedure for the extraction of the androgens. The results obtained in this and another study compare favorably with those reported by Oesting and Webster with sulfuric acid.

The results reported below were obtained from assays on complete 24-hour specimens of urine collected every third or fourth day for periods varying from 2 to 5 weeks. It is fairly well established that the 24-hour

[1] Publication I, "Studies of Growth of Private School Children." These studies were made possible by a grant from the General Education Board and by the cooperation of a private day school, Cambridge, Mass. The authors are indebted to Miss Frances Broughton and Miss Penelope Hill for assistance in securing the specimens of urine and for the creatinine determinations.

urinary excretion of creatinine depends upon the amount of muscle in any given individual and is rather constant from day to day. Consequently, creatinine determinations were made with the Evelyn photoelectric colorimeter when possible to verify the accuracy of the 24-hour urine collection.[2] It can be seen in the tables presented below that those androgen values (indicated by a superior figure) which vary markedly from the mean for the individual are associated with abnormal creatinine value. These therefore cannot be taken as the true excretion for the 24-hour period since it must be inferred that the collection was inaccurate. If the abnormal creatinine values are altered to conform to the mean value for the individual, and the corresponding androgen values are changed on this basis, it can be seen that the latter will more nearly approach those in which the collections were accurate. These children of both sexes represent average normals. They were chosen from a group of normal private school children whose growth was being extensively studied.

The tables shown below demonstrate that there are variations in the daily output of the androgens for any given child. In some instances, however, the values tend to be uniform, particularly in the youngest children. For the most part, the discernible variations in the individual case give no suggestion of a cycle. These fluctuations are not as great as those reported by Gallagher (12) et al. and Dingemanse et al. in normal adult males and females. Consequently, they cannot be considered to be significant, since the values are lower than in adults and there are no variations as much as 100 per cent in the accurately collected specimens of any child.

Male 75, age 6 yr. 3 mo.; height 119.4 cm.; weight 49.8 lb.

Date	*Androgen*	*Creatinine*
6-20-38	7.8	342
6-23-38	6.5	252
6-27-38	7.8	407
6-29-38	4.0	280
Mean	6.5	320

Male 1, age 8 yr. 1 mo.; height 134.3 cm.; weight 64.5 lb.

Date	*Androgen*	*Creatinine*
5-12-38	7.5	326[1]
5-15-38	6.0	375[1]
5-17-38	9.1	494
5-21-38	8.2	561
5-23-38	9.7	635
5-26-38	9.9	566
6- 5-38	6.8	552
6- 9-38	8.4	605
Mean	8.6	569

Male 43, age 10 yr. 11 mo.; height 152.1 cm.; weight 93 lb.

Date	*Androgen*	*Creatinine*
4-24-38	18.0	901
4-27-38	8.0	320[1]
5- 1-38	20.0	905
5- 5-38	16.5	810
5-10-38	22.0	940
5-13-38	4.0	286[1]
5-16-38	15.3	915
5-19-38	14.0	762
Mean	17.7	872

Male 16, age 12 yr. 2 mo.; height 157.3 cm.; weight 122 lb.

Date	*Androgen*	*Creatinine*
4-27-38	24.5	890
5- 1-38	21.3	1085
5- 4-38	31.0	1028
5- 9-38	23.3	1085
5-12-38	19.3	880
5-15-38	18.3	895
5-18-38	26.1	1110
5-21-38	18.7	915
Mean	22.6	986

[2] Studies of the creatinine excretion of these children are being carried out by N. Talbot and A. Stewart and will be reported in subsequent publications in this series.

Female 10, age 7 yr. 7 mo.; height 126.2 cm.; weight 55.5 lb.

Date	Androgen
5–13–38	7.5
5–15–38	8.5
5–19–38	4.5
5–23–38	4.5
5–26–38	6.0
5–29–38	6.3
6– 5–38	4.5
6– 8–38	4.5
6–12–38	7.0
6–16–38	6.0
Mean	5.5

Female 74, age 8 yr. 8 mo.; height 121.8 cm.; weight 60.8 lb.

Date	Androgen	Creatinine
3–31–38	10.0	535
4– 3–38	7.0	683
4– 6–38	8.5	430[1]
4–10–38	8.5	520
4–13–38	8.5	580
4–17–38	6.0	600
4–20–38	7.0	580
4–24–38	6.0	522
4–27–38	7.0	551
5– 1–38	9.5	658
Mean	7.7	581

Female 46, age 10 yr. 5 mo.; height 141.9 cm.; weight 66 lb.

Date	Androgen	Creatinine
4–18–38	6.0	500
4–20–38	9.0	663
4–23–38	7.8	657
4–27–38	7.0	610
5– 1–38	6.8	545
5– 4–38	6.3	632
5– 8–38	5.5	480
5–12–38	9.2	591
Mean	7.2	585

Female 15, age 11 yr. 5 mo.; height 142.2 cm.; weight 70.3 lb.

Date	Androgen	Creatinine
4–24–38	14.6	785
4–27–38	11.0	610
5– 1–38	16.8	696
5– 5–38	11.3	754
5– 9–38	19.3	
5–12–38	14.0	615
5–15–38	14.0	
5–18–38	15.8	
5–22–38	17.2	
Mean	14.9	692

Female 6, age 11 yr. 8 mo.; height 161.3 cm.; weight 109.5 lb.

Date	Androgen	Creatinine
4–27–38	10.0	525[1]
5– 1–38	10.3	660[1]
5– 4–38	17.3	1058
5– 9–38	15.5	749
5–12–38	8.5	490[1]
5–15–38	13.0	956
5–18–38	12.0	948
5–21–38	16.1	1410
Mean	14.8	944

Female 52, age 12 yr. 5 mo.; height 154.3 cm.; weight 96.5 lb.

Date	Androgen	Creatinine
5– 1–38	17.0	830
5– 5–38	18.0	925
5– 9–38	10.0	970
5–12–38	12.0	915
5–15–38	15.4	855
5–18–38	12.0	834
5–21–38	15.5	935
5–23–38	14.9	840
5–26–38	15.0	893
6– 2–38	17.4	856
Mean	14.7	885

[1] —Incomplete specimens.
Mean value—calculated with omission of incomplete specimens.
Androgen—International Units per day.
Creatinine—milligrams per day.

It can also be seen that the excretion of the androgens increases with age, males of the same age having a slightly higher excretion than females. One hundred and eighty-one androgen determinations on the urine of 87 children in our group indicate that the excretion also varies with the physical age as suggested by Dorfman and Gruelich, that is 2 children of the same chronological age, but of different physical development, may show significant differences in excretion. There is no evidence of a cyclic excretion of the androgens in either sex, which is also in accord with results in adults.

The facts outlined above, therefore, must be borne in mind when studying the sex hormonal output of normal children and obviously those with endocrine dysfunction and disease. Values obtained by us in a study of the latter groups revealed marked differences in the excretion of androgens when compared to the levels obtained for any given age group, (estimated from the chronological and physical age). It is apparent, therefore, that only a few assays for urinary androgens are necessary to obtain sufficient information concerning abnormal endocrine states.

SUMMARY

A study of the daily urinary excretion of androgens with a colorimetric method in girls and boys of varying age groups reveals the following.

There is a definite variation in androgen excretion from day to day but this is not of an adequate magnitude to be considered physiologically significant. Young children show less variation than the pre-adolescent or adolescent group.

There is no evidence of a cyclic excretion of the androgens. The excretion of androgens varies directly with increasing chronological age, and also with the physical maturity of the individual. There is a general level of excretion for any given age group when the above factors are taken into consideration.

The excretion of androgens in male children is slightly higher than in females of the same age and size. The androgen assay of a 24-hour urine specimen should yield significant information in studies of endocrine abnormalities of childhood.

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SUPPRESSIVE ACTION OF TESTOSTERONE PROPIONATE ON MENSTRUATION AND ITS EFFECT ON VAGINAL SMEARS[1]

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IN A PREVIOUS article (1) we reported the inhibitory effects of male sex hormone on human menstruation. It was shown that the administration of 775 mg. of testosterone propionate, oreton,[2] over a period of 31 days produced a temporary suppression of the menstrual cycle in a young woman. The treatment was followed by a series of changes in the vaginal secretion culminating in a smear picture comparable to that observed in the menopause (2). Further unpublished studies have shown these smear changes to be characteristic.

Since this hormone gives promise of being useful in dealing with human menstrual disturbances, and the information afforded by vaginal smears is so specific and readily obtained, a more detailed description of the smear changes occurring during and after treatment with this hormone will be given. This method of regulating hormonal therapy should prove as useful when the male sex hormone is given as it has been in guiding therapy with the estrogens (2).

EXPERIMENTAL PROCEDURE

The patient was admitted to the psychiatric clinic complaining of difficulty in concentration, loss of interest in her surroundings, tenseness, dysmenorrhea, menorrhagia and depression of spirits becoming most marked one week before the onset of each menstrual period and becoming less severe with the beginning of the menstrual flow. She was 18 years of age. Menstruation had begun at 13, occurred usually at intervals of 30 days and was fairly regular. Bleeding tended to be profuse. Pelvic examination showed negative findings.

She was kept under observation for 2 months before any endocrine treatment was given. During this period she was tense, depressed and had difficulty in concentration. The day of admission was the 27th day of the menstrual cycle. On the 30th day she had moderate bleeding which lasted 3 days. The next period began after the 36th day and lasted 7 days. Vaginal

[1] This study has been aided by grants from the Committee for Research on Sex Problems of the National Research Council and the Josiah Macy Jr. Foundation.

[2] We are indebted to Dr. E. Schwenk and Dr. G. Stragnell of the Schering Corporation for the supply of testosterone propionate (oreton) and estradiol benzoate (progynon-B).

smears were started with the onset of the following menstruation which occurred after an interval of 32 days.

Examination of the smears showed a normal menstrual phase with relatively profuse bleeding. The follicular reaction, though slightly weak, was fairly well expressed from the 8th to the 11th day (fig. 1). From the 12th through the 21st day 2000 R.U., and from the 22nd through the 25th day 1000 R.U. of estradiol benzoate (progynon-B) were administered intramuscularly for the purpose of regulating the bleeding and possibly relieving the dysmenorrhea and the mental depression. On the 20th day of the period (9th day of treatment with progynon-B) the vaginal smear showed a typical leucopenic smear with many small nucleated cornified cells, indicating a strong effect from the treatment (fig. 2). This smear which represents an induced follicular phase shows a considerable enlargement in the size of the cells and a more pronounced cornification as indicated by the greater eosinophilic staining reaction in comparison with the smear illustrated in fig. 1 which represents a normal follicular phase.

The next menstrual flow appeared after the 28th day, that is 4 days after the last injection with progynon-B, and lasted 8 days but was less profuse. A follicular phase followed from the 11th to the 13th day. Treatment with progynon-B was repeated from the 14th to the 23rd day with a daily dose of 2000 R.U. These two series of injections were followed by shortening of the interval, some decrease in dysmenorrhea and slightly less mental depression and concentration difficulty.

The next menstrual bleeding appeared after an interval of 25 days and lasted 6 days. It was followed by a follicular reaction from the 7th to the 10th day (fig. 3) comparable to the one before treatment was instituted (fig. 1). From the 12th to the 22nd day, which was the last day of this cycle, 25 mg. of testosterone propionate (oreton) was injected intramuscularly every other day. Vaginal smears during this period showed no deviation from the normal type. Fig. 4 shows a smear on the 22nd day of the menstrual period (11th day of treatment with oreton). This is of premenstrual type and comparable to a normal premenstrual smear with bacillus vaginalis. This bacillus when present produces a characteristic smear with very marked cytolysis. The next period appeared after the short interval of 22 days. Although it lasted 9 days including 3 days of spotting, flow was scantier than during the preceding periods. Oreton was continued in doses of 50 mg. on the 2nd, 4th and 6th day, 75 mg. on the 8th, 10th, 12th, 14th and 16th day, and 50 mg. on the 18th and 20th day.

The first deviation from the normal vaginal smear was noted on the 6th or 7th day of the period (17th or 18th day from beginning of treatment).[3] Fig. 6 illustrates a smear taken on the 8th day of bleeding (18th day of treatment). The appearance of smaller and rounded cells is clearly shown. In fig. 5, a smear taken on the 3rd day of menstruation (14th day of treatment), the small rounded cells are still absent.

[3] In our previous paper (1) an error was made in compiling the figures for days from beginning of treatment as given in parentheses. Correction should be made by adding 3 days to these figures.

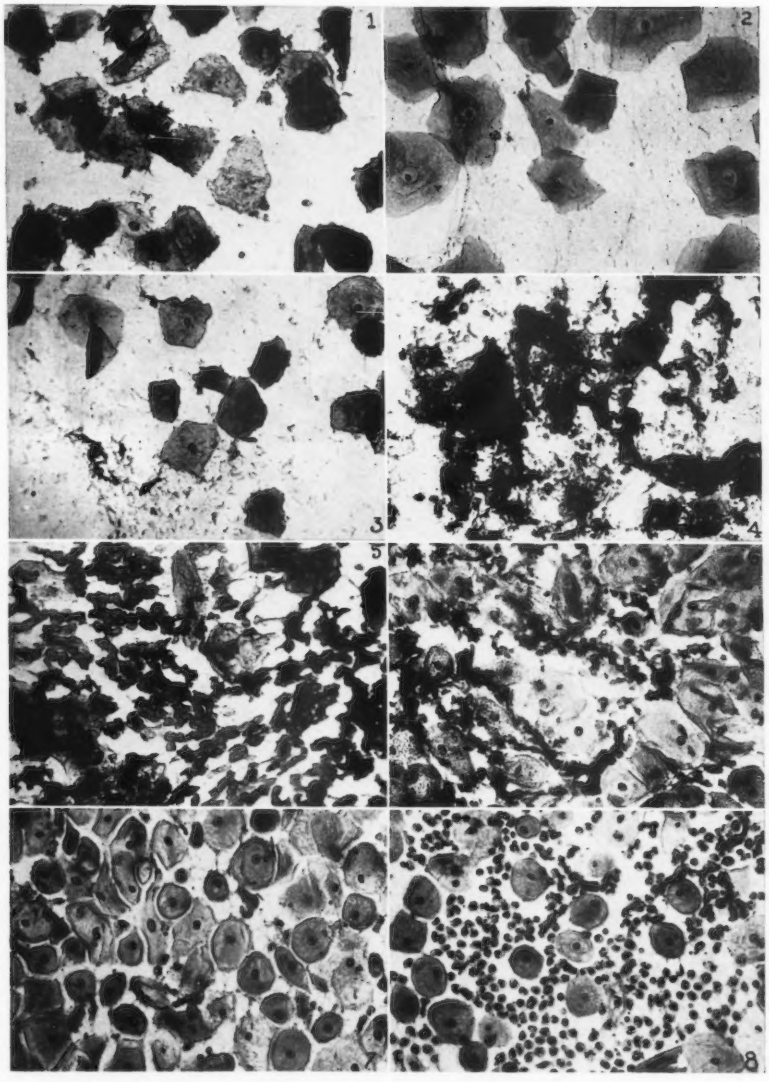

Fig. 1. Normal follicular phase before treatment. 9th day. Fig. 2. Follicular phase induced with progynon-B. Fig. 3. Follicular phase. 10th day. Fig. 4. Premenstrual phase with bacillus vaginalis and pronounced cytolysis. 22nd day (11th day of treatment with oreton). Fig. 5. Menstrual phase. Vaginal cells are of the large squamous type. 3rd day (14th day of treatment with oreton). Fig. 6. Late menstrual phase. Appearance of rounded deep cells. 7th day (18th day of treatment with oreton). Fig. 7. Transitional (descending) smear type showing predominance of rounded compact cells and relative leucopenia. ('Round cell type'). 11th day (22nd day of treatment with oreton). Fig. 8. Early atrophic or menopausal type with round cells and pronounced leucocytosis. (Type 1). 12th day (23rd day of treatment with oreton.) Fig. 1 to 8 ×180.

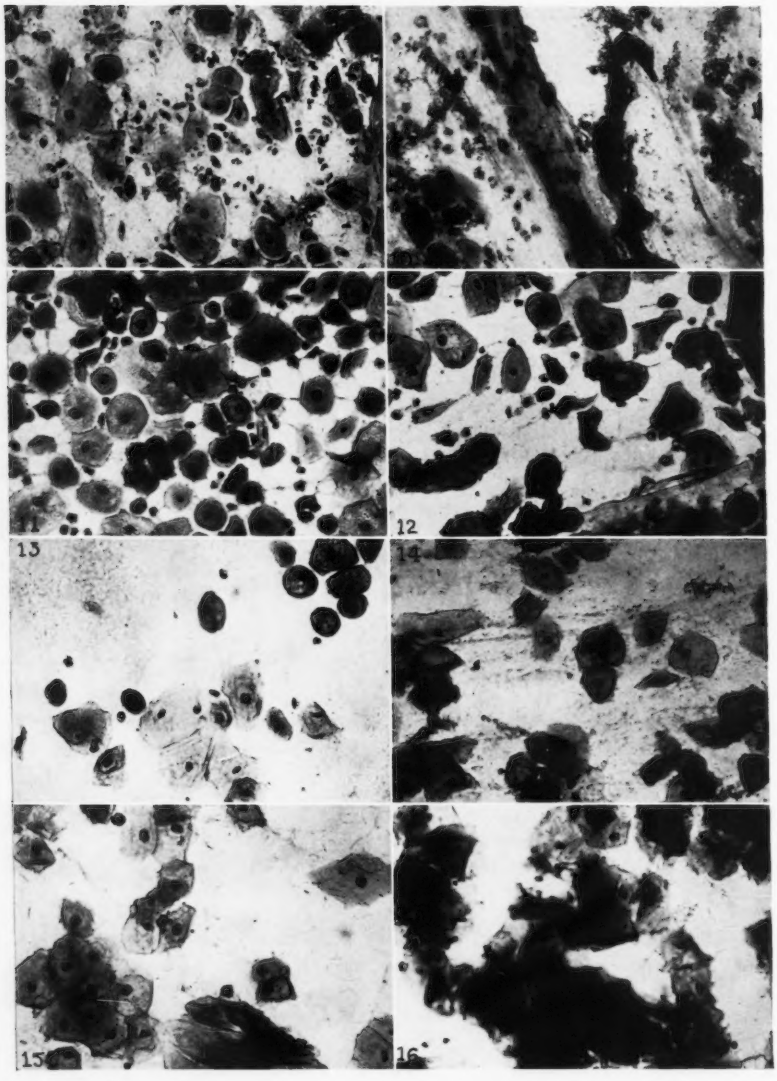

Fig. 9. ADVANCED ATROPHIC OR MENOPAUSAL TYPE (Type 2). 20th day (31st day of treatment with oreton). Fig. 10. SMEAR TYPE WITH EXCESSIVE MUCUS AND FIBRINATION. (Withdrawal reaction.) 28th day (8 days after discontinuation of oreton). Fig. 11. TRANSITIONAL (ASCENDING) 'ROUND CELL TYPE' COMPARABLE TO THE ONE ILLUSTRATED IN FIG. 7. 35th day (15th day after discontinuation of oreton). Fig. 12. REAPPEARANCE OF LARGE CELLS IN INCREASING NUMBERS. 37th day (17th day after discontinuation of oreton). Fig. 13. TRANSITIONAL SMEAR SHOWING APPEARANCE OF CORNIFIED CELLS WITH PYKNOTIC NUCLEI. (Weak follicular reacton.) Fig. 14. NAVICULAR STAGE. 39th day (19 days after discontinuation of oreton). Fig. 15. FURTHER ENLARGEMENT OF CELLS AND REAPPEARANCE OF BACILLUS VAGINALIS. 40th day (20 days after discontinuation of oreton). Fig. 16. PREMENSTRUAL TYPE OF SMEAR WITH BACILLUS VAGINALIS. (cytolysis less pronounced than in Fig. 4.) Fig. 9 to 16 ×180.

In subsequent smears there was a gradual replacement of the large squamous epithelial cell types by smaller, rounded, more compact cells with larger nuclei. On the 11th day of the period (22nd day of treatment) a characteristic and significant type of smear relatively leucopenic and rich in cells predominantly of the small rounded type appeared (fig. 7). The sudden appearance of many new leucocytes characterized the smear (fig. 8) of the following day (23rd day of treatment). This type of smear appeared as a transition into the atrophic or menopausal smear type. It might be designated as 'atrophic (menopausal) type I.' It was followed by a gradual change into the more advanced atrophic or menopausal type, which prevailed throughout the remaining period of treatment and for approximately 5 days after treatment was stopped (fig. 9). Rounded deep cells continued to predominate. Leucocytosis was less marked. Scattered erythrocytes, which are often found in atrophic smears, were frequently present.

During the menstruation which occurred after beginning treatment with oreton, the usual sharp abdominal cramps which had characterized previous dysmenorrhea were replaced by more generalized, less severe abdominal discomfort and pains in the back, groin and legs. After bleeding had ceased and during the remainder of the treatment the patient had sensations as though she were about to menstruate. Mentally she was more alert and cheerful than she had been at any time since admission.

On the 26th day (6 days after cessation of treatment) there was an excess of mucous secretion accompanied by fibrinated blood. There was no gross bleeding. This reaction lasted for 4 to 5 days (fig. 10). The smears retained their menopausal character with leucocytes gradually becoming less numerous particularly after the 31st day (11th day after cessation of treatment). On the 35th day of the period (15th day after cessation of treatment) a cellular and leucopenic type of smear consisting chiefly of rounded cells was observed (fig. 11). This very characteristic stage is comparable to the smear illustrated in fig. 7. Both of these smears represent the same transitional stage from either the normal to the menopausal (fig. 7) or the menopausal to the normal (fig. 11).

After this stage large cells began to reappear in increasing numbers and to replace the small round cells. This is shown in fig. 12 which illustrates the smear on the 37th day (17th day after cessation of treatment). Cornified cells with pyknotic nuclei of the type prevailing during the follicular phase were also noted. A group of such cells is shown in fig. 13 which illustrates the 38th day of the period (18th day after cessation of treatment). A typical follicular phase was absent though cells of the follicular type appeared at this time along with cells of other types including small rounded cells. On the 39th day (19th day after cessation of treatment) another characteristic stage appeared. The small rounded cells had been replaced by slightly larger cells many of which were of navicular form (fig. 14). On the 40th day (fig. 15) the enlargement of the cells was more apparent and the bacillus vaginalis which had disappeared during the induced atrophic phase were again seen.

A premenstrual smear with large squamous cells prevailing was noted on the 45th day (25th day after cessation of treatment) (fig. 16). There was an absence of the pronounced cytolysis noted in fig. 4. At the end of the 48th day, that is, 28 days after the last injection of oreton, menstrual flow began with a moderate amount of bleeding which lasted 8 days. It was followed by a weak follicular and then by a normal premenstrual phase. The next menses appeared after the 31st day.

Following the improvement noted above in the mental condition of the patient she became more irritable and depressed. During the 2 menstrual periods which occurred after the administration of oreton there was a moderate amount of dysmenorrhea. No increase in hair growth, deepening of the voice or change in the external genitalia were noted during or after the treatment with oreton.

DISCUSSION

The inhibitory action of the male hormone on human menstruation, which is illustrated by this case, is in accord with previous studies on lower mammals and primates (3, 4, 5, 6). The absence of uterine bleeding and the persistence of an atrophic vaginal smear type during the period of treatment with the male hormone, as described above, indicate that the cyclic uterine and vaginal functions were temporarily suppressed.

Experiments in human beings are as yet too meagre to permit any conclusions as to the exact sequence of events by which these effects on the uterus and vagina are brought about. The effects of the male hormone in animals are better understood. In the ovaries there is an inhibition of maturation of the follicles and of ovulation (4) with a subsequent cessation of cyclic uterine and vaginal activity.

Whether the male hormone acts directly on the ovaries or indirectly through its action on other endocrine glands is still uncertain. Recent experiments in mice (7) and monkeys (8) showed that gonadotropic principles are capable of inducing ovarian activity despite the concomitant injection of amounts of testosterone propionate which ordinarily suffices to inhibit the cycle. These findings could be interpreted as indicating that the suppression of ovarian function by the male hormone depends on its inhibitory effect on the gonadotropic activity of the hypophysis.

Another mode of action of the male hormone has been pointed out by Robson (7) in that it is capable of preventing the vaginal cornification usually induced by estrogens in castrate mice. This presumably peripheral neutralization of the effects of estrogens by androgens on vaginal cornification has also been demonstrated in women (9).

That menstrual functions can be inhibited in human beings by the administration of the male hormone, with the induction of a temporary sterility, is of considerable therapeutic significance. A beneficial effect upon excessive and uncontrolled uterine bleeding has already been reported (10, 11). This cessation of bleeding was associated with the development of an atrophic endometrium (10), an observation which has been subsequently confirmed (11, 12). This endometrial atrophy is the uterine counterpart of

the changes in the vaginal epithelium which underlie the atrophic vaginal smear we have observed to follow the administration of the male hormone. The hormone also appears to be of therapeutic help in the treatment of the menopausal syndrome (9, 13, 14).

With the increasing use of the androgenic hormones in women, there is an evident need for simple methods for guiding therapy such as was made available for the estrogens by the vaginal smear changes which follow the administration of the latter group of hormones (2). With the estrogens, the vaginal smears, with adequate therapy, were found to undergo a series of characteristic changes from the initial menopausal type culminating in a follicular type of smear, characterized by leucopenia and by cornified squamous cells with pyknotic nuclei. The presence of progressive intermediate changes permitted a quantitative evaluation of the extent of replacement therapy.

In the case of the androgens, the vaginal smear changes we have described promise to afford an equally accurate index for the use of these hormones as it has for the estrogens. The changes through which the vaginal secretions go appear to be extremely consistent. The end stage is equally well marked. The final stage is that of the atrophic smear such as is found in the menopause and in the primary amenorrheas (2) or in the pre-pubertal state (15). The squamous superficial cells characteristic of the active menstrual cycle are gradually replaced by small rounded compact cells. Between these extremes occur a number of intermediate stages of a regressive character which permit a quantitative estimate of the extent of effect obtained. With the discontinuation of therapy there is a reversal of the smear change in the direction of the normal picture, with a final resumption of normal follicular activity.

It is of interest that a withdrawal reaction appeared approximately 6 days after the discontinuation of treatment with the male hormone. This consisted in the appearance of much mucus and many erythrocytes. It corresponds in the time of appearance to the withdrawal reaction which follows the discontinuation of estrogenic therapy (2), at which time there is an increased mucous secretion and a pronounced bleeding.

Another advantage offered by the vaginal smear in the treatment of women by androgens lies in the fact that it should prevent overdosage. Once the atrophic smear is reached, the dose can be reduced to the minimal one which will maintain it. While no masculinizing effects of the male hormone were noted in this one patient, a recent study has reported changes such as enlargement of the clitoris, marked vulval hyperemia and temporary lowering of the voice with intensive androgenic therapy (9).

SUMMARY

Testosterone propionate inhibited the menstrual cycle of a young menstruating woman. Menstruation was resumed spontaneously 28 days after the cessation of therapy. Examination of the vaginal smears was made during the various phases of the menstrual cycle. With the administration of

the male hormone a transition to an atrophic menopausal type of smear took place. With the cessation of therapy the smears returned to normal. These definite changes in the vaginal secretion offer a simple and reliable method for evaluating the effect of the male hormone in women.

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THE TREATMENT OF FEMALE MENOPAUSE WITH MALE SEX HORMONE

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THE TREATMENT of menopause in the female was a difficult problem until the discovery of female sex hormone and its value. Male sex hormone has only recently been available in sufficient amount to permit its use therapeutically.

Butenandt (1), Koch (2), and others have reported that some of the unsaturated androgens, notably androstenedione and dehydroandrosterone, will induce estrus in the normal infantile rat. Browman (3) reported the cessation of estrous cycles in adult normal rats injected with androsterone and testosterone. Nelson and Gallagher (4) found that androstanediol and androstenedione failed to produce vaginal cornification, but did induce uterine and mammary hypertrophy and prevented castration changes in the hypophysis. Testosterone and androstanedione induced definite mammary proliferation and enlargement of the uterus while androsterone and dehydroandrosterone failed to produce such changes. Trans-androstenediol failed to influence the cycles or to significantly affect the ovaries, mammary glands or uteri. Cis-androstenediol induced markedly prolonged periods of estrus in adult female normal rats, while dehydroandrosterone produced even longer periods of estrus. The administration of dehydroandrosterone to hypophysectomized females produced typical estrous smears for a prolonged period. The ovaries were only slightly decreased in size, the mammary glands were not stimulated, and the uteri were enlarged.

Robson (5) observed abolishment of estrous cycles in mature female mice during two weeks while giving testosterone, and the reestablishment of the cycles following cessation of injections. Mature ovariectomized mice were injected with estrone in doses sufficient to produce cornification, together with testosterone; there was a resultant inhibition of the estrous reaction of the vagina.

McEuen, Selye and Collip (6) found that testosterone in doses sufficient to produce mammary gland development did not cause vaginal cornification either in the normal or the ovariectomized immature female rat. Magistris (7) found it possible to produce temporary sterility during injection of testicular substance in adult female mice. His results were irregular, and not in direct relationship to the amount of substance used. Cohen-Salol and Courrier (8) determined that even 20 mg. of testosterone acetate did not produce an es-

trous effect on the castrated female, but that this amount of testosterone had an inhibitory effect on dihydrofolliculin when injected simultaneously.

Papanicolaou, Ripley, and Shorr (9) presented a case in which the administration of testosterone propionate interrupted regular menstrual cycles in a young woman. The vaginal smears showed that during the period of induced amenorrhea there was a replacement of the normal smear picture by an atrophic menopausal type. This was then followed by gradual resumption of follicular activity and a normal smear picture. Robson (5), Hartman (10), and Zuckerman (11) have demonstrated the antagonistic action and suppressive effect of male sex hormone on ovulation and menstruation.

Mocquot and Moricard (12) found that administration of testosterone acetate modified and in general ameliorated the functional disorders following castration in women, but had no effect on the trophic vaginal disturbances; occasionally nervous instability occurred, but frequently a sensation of euphoria. The amounts they gave were small (5 mg.) and the frequency about every 7 to 14 days. It is our feeling that their failures were due to insufficient dosage, the wonder being that they obtained good results at all. Salmon (13) used testosterone propionate on a castrated woman and noted relief of menopausal disturbances with a total dose of 400 mg. over a period of 30 days. Vaginal smears during relief from symptoms showed a full estrogenic effect equal to that produced by previous treatment with 80,000 I.U. of estradiol benzoate. There was corresponding absence of gonadotropic hormone. Margiel and Zwilling (14) reported several cases of female menopause treated with moderate success by injection and tablets of male sex hormone.

We are reporting a series of 21 cases of female menopause, both natural and artificial, treated by the exhibition of male sex hormone, in the form of testosterone propionate in oil,[1] administered by injection intramuscularly. Since there is a close chemical relationship between the female and male sex hormones, and because of the finding of both female and male sex hormone in normal women, we believed it possible to obtain relief from symptoms of menopause by means of male sex hormone. Animal experimentation has shown the benefits of administration of male sex hormone to the castrate female.

The patients in this series were suffering from headaches, flushes, and sweats, the usual symptoms of menopause, in addition to nervous upsets, and other symptoms which will be considered later. In all of these women the symptoms were severe or very severe; usually these women would find relief only by the administration of female sex hormone as given in our routine method for severe cases—50,000 I.U. 2 or 3 times weekly, followed by a gradual tapering off to a dose of 10,000 I.U. twice weekly. Naturally, in beginning treatment with male sex hormone, it was impossible to gauge satisfactorily the dosage necessary, but in a short time, a very definite com-

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[1] We wish to express our appreciation to the Schering Corporation for their courtesy and cooperation in supplying male sex hormone in the form of testosterone propionate.

parison was found. We learned that it was best to begin treatment with the exhibition of 25 mg. of testosterone propionate twice weekly—this was followed by improvement of the patients subjectively to the extent of at least 80 per cent within 2 to 3 weeks, after which time it was possible to control the symptoms almost perfectly by administration of 30 to 40 mg. of the hormone weekly, in divided doses. Due to the extreme difficulty in obtaining enough material to use conclusively on a large series of cases we had ample opportunity to find out whether our results were due to chance. When our weekly dose was cut down to 10 to 20 mg. in these severe cases, the patients promptly (usually after 2 weeks) had recurrences of their initial symptoms.

By the administration of 30 to 50 mg. of testosterone propionate we were able to ameliorate completely or almost completely the symptoms of menopause: headaches, flushes, and sweats. In our series of 21 cases, there was no case of failure, but many cases that appeared to be failures until we were able to give them the amount of hormone necessary. We noted no effect on the breasts, other than that no patient complained in any way of mastalgia. We found only one case where arthritis accompanying menopause was benefited, and we believe female sex hormone to be of more value in treating this condition, as we have had encouraging results in several cases of so-called menopausal arthritis. There were no instances of itching or discharge where we felt that the condition was due to the male sex hormone, nor was any case found where abnormal dryness of the vagina occurred which had not existed prior to treatment. In this regard, it may be said there were two cases of trichomonas vaginalis with pruritus, which responded to treatment with 25 per cent salt douches. Several of the younger women (age limit of the group of women treated was from 24 to 60 years) who had had a surgical menopause with resulting markedly severe symptoms responded fully as well as those in whom the menopause was spontaneous. One patient presented a very interesting problem. She had been surgically castrated at the age of 40, developing severe menopausal symptoms subsequently. On administration by intramuscular injection, of female sex hormone in oil, she broke out in a rather generalized rash, with an area of redness and swelling at the site of injection, about 15 cm. in diameter. There was considerable accompanying itching. The patient reacted in like manner to tablets given orally, and on skin testing, showed a definite sensitivity to estradiol benzoate, the preparation which we were using. This woman responded to testosterone propionate, without any reaction denoting sensitivity. Three cases had vaginal bleeding during the treatment with testosterone propionate, all being spontaneous menopause cases with irregularity of the menses for the previous period of 1 to 3 years. Because of the difficulty in getting these patients to come in at the onset of their bleeding, it was possible to obtain an endometrial biopsy in only one case; this revealed a resting phase with a rather scant endometrium. One patient, a severe diabetic, was relieved of her flushes and sweats, without any effect on her diabetic status. A few patients complained of localized pruritus on one oc-

casion only, at the site of injection. No effect was noted on hypertension. Three had been previously treated with large doses of estradiol benzoate after radiation menopause, with subsequent uterine bleeding; none of these women had any bleeding following the male hormone.

We are making special mention at this time of our treatment of a young woman, age 26, who underwent a hysterectomy and bilateral salpingo-oöphorectomy 7 months ago, for a papillary cyst-adenocarcinoma of the ovary without definite metastases throughout the abdomen. This patient was treated postoperatively with x-ray radiation, and had terrific menopausal disturbances within a very short time after operation. She received 9 injections of 5000 I.U. each of estradiol benzoate prior to our seeing her, at which time we advised discontinuance of the female sex hormone, and substitution of the male sex hormone. This woman is kept practically free from headaches, flushes, and sweats by administration of 40 to 50 mg. of testosterone propionate weekly. However, we hope to have available soon another means of treatment with the male sex hormone by which small amounts of the hormone will be absorbed by the body over a long period of time. This patient is now 7 months postoperative; she has gained 40 lb., but had lost 20 lb. prior to operation; she feels and looks well. Gain of weight has taken place in the usual castrate manner.

We are, at the present time, working on the effect of testosterone propionate on ovulation bleeding, and will report our results. We are also carrying out experimental work on rats to determine inhibitory effects on the castrated female by injection of male sex hormone.

SUMMARY

There is a definite place for male sex hormone (testosterone) in the treatment of female menopause. It is possible to control the symptoms of menopause by the weekly administration of 30 to 50 mg. of testosterone propionate. It is apparent that the exhibition of 20 to 40 mg. of the male sex hormone will not inhibit bleeding, even though the bleeding is not that found in normal menstruation accompanied by the formation of a functioning corpus luteum.

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PROGESTERONE IS ANDROGENIC

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SEVERAL WORKERS have shown that ovaries transplanted into a large series of castrate males (rats and guinea pigs) have an androgenic effect in an occasional animal (1, 2, 3). Hill (4, 5) has transplanted the ovaries of mice to the ear where they are subjected to a temperature several degrees lower than are abdominal ovaries. In this position, the ovaries apparently produce an androgenic substance. This work has been confirmed by Deansely (6) in the rat. Hill noted an extensive luteinization of the transplanted ovaries and Deansely has suggested that there may be a relation between thecal luteinization and the masculinizing action of the graft. Desclin (7), however, disagrees with this hypothesis. Papanicolaou (8, 9) has shown that ovaries in normal guinea pigs have an androgenic action when stimulated with the APL factor of pregnancy urine. He believes that this androgenic substance is produced by the hypertrophied interstitial tissue of the ovaries.

Progesterone is chemically similar to other known androgenic compounds. Nelson (10) has shown that progesterone is similar to androgens in that it will maintain spermatogenesis in hypophysectomized rats. Yet it has been stated that progesterone is not androgenic (11). Assays on rats (4) and on capons (12), using small amounts of progesterone, have given negative results. Hill (13) has shown, by biological methods, that the androgenic substance produced by the grafted ovaries is not testosterone. Since the ovary is known to produce progesterone and, under the conditions of the grafting and stimulation experiments, the ovary also possesses androgenic powers, it seemed of interest, particularly in view of the conflicting reports on this subject, to determine whether or not large amounts of this substance have androgenic powers. While the present work was in progress, a preliminary note (14) appeared in which androgenic effects on the sexual accessories of rats with synthetic progesterone and with extracts of corpora lutea were reported.

PROCEDURE AND RESULTS

Crystalline progesterone, synthetically prepared, and progestin, extracted from corpora lutea and standardized in international units, were injected subcutaneously into white rats. Both were administered in peanut

[1] We wish to thank Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products Inc. for the generous supply of crystalline progesterone, and Dr. Oliver Kamm of Parke Davis and Dr. E. Gifford Upjohn of Upjohn Corporation for the generous supply of standardized progestin used.
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oil. In all cases, the animals were killed on the day following the last injection. The entire genital tract was removed and fixed in Bouin's solution. After fixation, the ventral prostatic lobes and seminal vesicles were removed under a dissecting microscope and weighed. Histological preparations were then made. Results obtained were evaluated on the basis of comparison between treated and untreated (or oil control) litter mates.

Group 1. Five intact, 19-day-old male rats were given 2.0 or 3.0 mg. of crystalline progesterone daily for 5 days. The ventral prostates, in each of these treated animals, were heavier than the untreated litter-mate controls (table 1). While the seminal vesicles were larger in the treated animals than in the controls, the difference is not considered significant. It has been shown that the ventral prostate is more sensitive to androgenic stimulation than the seminal vesicle (15). Histological preparations showed cytological evidence of secretory function (Moore's 'clear area,' 16) in the

TABLE 1. INTACT IMMATURE RATS, PROGESTERONE TREATMENT

Litter No.	Age at autopsy days		Daily dose mg.	Days treatment	Total dose mg.	Wt. of prostates, mg.	Wt. of sem. ves., mg.
1	Treated	24	2.0	5	10.0	25.0	8.5
	Control	24				22.0	8.0
2	Treated	24	2.0	5	10.0	53.0	10.0
	Control	24				24.0	9.0
	Average of treated animals					39.0	8.5
	Average of control animals					23.0	8.5
3	Treated	24	3.0	5	15.0	25.0	9.0
	Treated	24	3.0	5	15.0	45.0	14.5
	Treated	24	3.0	5	15.0	32.5	13.0
	Control	24				20.0	8.0
	Control	24				20.0	9.0
	Control	24				18.0	11.0
	Average of treated animals					34.2	12.2
	Average of control animals					19.3	9.7

prostates of both treated and untreated animals in this group. The seminal vesicles showed no definite evidence of secretory function.

Group 2. All the animals in this group were castrated at 19 days of age. The treated animals were given daily injections of progesterone; 3.0 mg. daily for 20 days or 6.0 mg. daily for 10 days. Treatment was started the day of castration. The ventral prostates of the treated castrates showed a constant and marked increase in weight over those of the untreated castrate litter mates (table 2). The seminal vesicles of the treated animals also showed a weight increment. Cytological evidence of secretory function was found in the prostates of all the treated animals in this group (fig. 1). The prostates of the castrate controls killed at 39 days of age (litters 4 and 5) showed castration atrophy with no evidence of secretory function (fig. 2). The cytological findings in the castrate controls of the litter killed at 29 days of age were somewhat equivocal in that an occasional 'light area' was found. Similar findings in immature castrates under the age of 35 days have been reported (17). While the epithelium of the seminal vesicles was higher in the treated than in the untreated litter mates, clear evidence of function (secretory granules of Moore, 16) was not found.

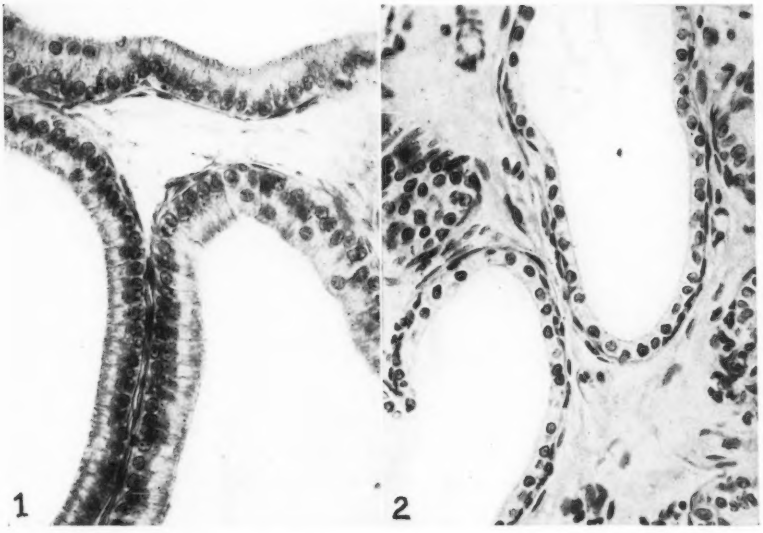

Fig. 1. Ventral prostate of progesterone treated immature castrate killed at 39 days of age. (Table 2, litter 5) ×320.
Fig. 2. Ventral prostate of control immature castrate killed at 39 days of age. (Table 2, litter 5.) ×320.

Group 3. The animals in this group were young adults castrated at 45 days of age. Treatment was begun 20 days later, at 65 days of age, and continued for 7 days. Daily dosages varied from 2.0 mg. to 35.0 mg. Table 3 shows that there was a definite increase in the weight of the ventral prostates and seminal vesicles with daily dosages of from 17.5 to 35.0 mg., even with this short period of treatment. Cytological

Table 2. Castrate immature rats, progesterone treatment. Castrated at 19 days

Litter No.	Age at autopsy days		Daily dose mg.	Days treatment	Total dose mg.	Wt. of prostates, mg.	Wt. of sem. ves., mg.
4	Treated	39	3.0	20	60.0	62.5	16.5
	Treated	39	3.0	20	60.0	69.0	14.0
	Control	39				11.0	5.0
	Control	39				14.0	5.0
5	Treated	39	3.0	20	60.0	82.0	15.0
	Treated	39	3.0	20	60.0	62.0	8.0
	Treated	39	3.0	20	60.0	70.0	12.5
	Control	39				12.0	5.0
	Control	39				10.0	5.0
	Average of treated animals					69.1	13.2
	Average of control animals					11.8	5.0
6	Treated	29	6.0	10	60.0	34.0	7.0
	Treated	29	6.0	10	60.0	53.0	7.5
	Control	29				14.0	4.5
	Control	29				20.0	5.0
	Average of treated animals					43.5	7.3
	Average of control animals					17.0	4.8

evidence of secretory function was found with dosages of 35.0, 17.5, 11.66 and 9.0 mg. daily, but none with dosages of 5.0 and 2.0 mg. daily. In the seminal vesicles, no secretory granules were found. However, in the seminal vesicles of animals receiving 35.0, 17.5, and 11.66 mg. daily, the villi were better developed than in the castrate controls. The epithelial cells of these glands were cuboidal to columnar in type, whereas the cells in the glands of the castrate controls were barely wider than their nuclei.

TABLE 3. ADULT CASTRATES, PROGESTERONE TREATMENT. CASTRATED AT 45 DAYS. TREATMENT BEGUN AT 65 DAYS

Litter No.		Age at autopsy, days	Daily dose, mg.	Days treatment	Total dose, mg.	Wt. of prostates, mg.	Wt. of sem. ves., mg.
	Treated	72	35.0	7	245.0	62.0	40.0
7	Treated	72	17.5	7	122.5	42.0	34.0
	Treated	72	11.66	7	81.62	28.0	24.0
	Control	72				22.0	21.0
8	Treated	72	9.0	7	63.0	39.0	49.0
	Control	72				36.0	46.0
	Treated	72	5.0	7	35.0	17.0	22.0
	Treated	72	5.0	7	35.0	20.0	28.5
9	Control	72				16.0	29.0
	Control	72				17.5	25.0
	Oil control	72	0.2 cc. oil	7		18.0	29.0
	Oil control	72	0.2 cc. oil	7		17.5	31.5
	Treated	72	2.0	7	14.0	26.0	24.5
10	Treated	72	2.0	7	14.0	17.0	20.5
	Control	72				17.0	22.5

Group 4. The animals in this group, both immature and adult castrates, were given progestin (extract of corpora lutea in oil) in daily dosages of 1.0 to 3.0 I.U. Some of the castrate controls were given equivalent doses of peanut oil. The immature animals were castrated at 19 days of age and treated for 20 days. The adults were castrated at 45 days of age, treatment was begun 20 days later and continued for 7 days. Table 4 shows that there was a marked increase in the prostatic weights of the treated immature castrates. An increase in seminal vesicle weight was also obtained. In the adult castrates, with the dosages used (1.0 to 2.0 mg. daily), no clear cut evidence of stimulation was observed. Much higher dosages would probably be effective, but were not tried since the most concentrated progestin solution made available to us contained only 10 I.U. per cc. Dosages of progestin equivalent to 35.0 mg. of progesterone would necessitate the administration of impossible amounts of oil. Cytological evidence of prostatic function was found in all the treated immature castrates, but not in the untreated immature castrates nor in the treated or untreated adult castrate animals. No cytological evidence of secretory function in the seminal vesicles was found in either the treated or control castrate immatures or adults.

Group 5. Four new-born females of 2 litters were injected with 0.5 mg. of progesterone. Treatment was continued daily with gradually increasing doses until the 30th day of life. Three animals each received a total dosage of 45.0 mg. and one animal received 73.0 mg. The clitoris of each of these animals was approximately three times as large as the clitoris of the 2 untreated controls. Pressure on the prepuce caused an eversion of the enlarged clitoris in the treated rats. No eversion could be produced in the untreated litter mates nor in normal females of any age.

TABLE 4

Litter No.	Age at autopsy, days	Daily dose, I.U.	Days treatment	Total dose, I.U.	Wt. of prostates, mg.	Wt. of sem. ves., mg.
Castrate immature rats, progestin treatment. Castrated at 19 days						
	Treated 39	3.0	20	60.0	74.0	13.0
13	Treated 39	3.0	20	60.0	58.0	14.0
	Oil control 39	0.3 cc. oil	20	6.0 cc. oil	14.0	10.5
	Average treated animals				66.0	13.5
	Average control animals				14.0	10.5
	Treated 39	2.0	20	40.0	75.0	14.5
12	Treated 39	2.0	20	40.0	42.5	10.0
	Oil control 39	0.2 cc. oil	20	4.0 cc. oil	10.0	7.5
	Oil control 39	0.2 cc. oil	20	4.0 cc. oil	12.0	7.0
	Average treated animals				58.8	12.3
	Average control animals				11.0	7.3
	Treated 39	1.0	20	20.0	46.0	10.0
	Treated 39	1.0	20	20.0	41.0	9.5
11	Treated 39	1.0	20	20.0	57.5	9.5
	Oil control 39	0.1 cc. oil	20	2.0 cc. oil	10.0	6.5
	Oil control 39	0.1 cc. oil	20	2.0 cc. oil	8.5	7.5
	Average treated animals				48.2	9.7
	Average control animals				9.3	7.0
Castrate adults, progestin treatment. Castrated at 45 days, treatment started at 65 days						
	Treated 72	2.0	7	14.0	19.0	20.0
10	Treated 72	2.0	7	14.0	27.5	29.0
	Treated 72	1.0	7	7.0	15.0	21.0
	Control 72				17.0	22.5

DISCUSSION

Relatively enormous quantities of progesterone and of progestin are necessary to evoke an androgenic effect. Since these substances are costly, the number of experimental animals in any one group is not large. The data amassed indicate, however, that these substances are qualitatively androgenic in that they maintain or stimulate male accessory glands, not only as to weight, but also as to secretory function. No attempt has been made to establish the quantitative androgenic power of progesterone nor of progestin from natural sources.

In all groups in which treatment was started immediately after castration, the prostates of the treated animals were larger than those of the controls. The maximum increase was obtained in litters 4 and 5 (table 2). These animals were given 60.0 mg. of progesterone over a 20-day period (2 mg. daily). The average weight of the prostates of the treated animals was 5.85 times that of the control litter mates. Cytological evidence of secretory function was not found in any of the controls with the exception of the castrate controls killed at 29 days of age (litter 6).

It is a well established fact that the prostates respond more readily to lower doses of androgen than do the seminal vesicles. This fact probably explains why the weight response of the seminal vesicles was less than that shown by the prostates, and in some cases was equivocal. However, in

litters 4 and 5 (table 2), the seminal vesicles weighed 2.64 times those of their untreated castrate litter-mate controls. The cytological responses of the seminal vesicles was not as clear cut as in the prostates. 'Secretory granules' were not found, but usually there was a difference in height of the epithelium of the glands, that of the treated animals being higher than that of the controls.

That progesterone will repair as well as prevent castration changes is evident from the fact that dosages of 9.0 to 35.0 mg. daily given for 7 days to animals which had been castrated 20 days before treatment was started (litters 7 and 8, table 3), produced definite cytological evidence of secretory function in the ventral prostates. An increase in weight of the gland was also evident even with such a short period of treatment. The prostate of the animal given 35.0 mg. daily weighed 2.81 times that of the control, and the prostate of the animal given 17.5 mg. daily was 1.9 times that of the control.

On the basis of the few animals used, no great difference was noted between the androgenic effects of a milligram of progesterone and its equivalent, an I.U. of progestin. For example, the average prostatic weight of the animals receiving 3.0 mg. of progesterone daily for 20 days was 69.1 mg., while that of animals receiving 3.0 I.U. of progestin for the same period was 66.0 mg.

Administration of androgens to the female rat causes enlargement of the clitoris. Enlargement of the clitoris is also obtained when large amounts of APL are administered to the intact guinea pig, but is not obtained when the animal is ovariectomized (9). This is presumptive evidence that the APL causes the ovary to produce an androgen. Experiments in progress indicate that a similar enlargement of the clitoris may be obtained in the rat by administration of large amounts of APL. Experimental results presented in this paper (group 5) show that large amounts of progesterone also effect clitorine enlargement.

Progesterone, then, simulates the androgenic effects of transplanted ovaries and of ovaries stimulated with large amounts of APL. What particular androgen is produced by the ovaries under these circumstances is not known. The experimental data presented do not warrant the conclusion that the androgenic activity of these ovaries is due to their production of large amounts of progesterone. However, in view of the evidence, it is possible, at least, that part of the androgenic power of these ovaries may be due to the progesterone produced.

SUMMARY

Large amounts of progesterone and of progestin given to castrate male rats maintain or stimulate the prostates and, to a lesser extent, the seminal vesicles. These sexual accessories increase in weight with such treatment and display cytological evidence of secretory function. An increase in size of the clitoris can also be obtained by administration of progesterone. From these facts, it is concluded that progesterone is androgenic.

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CREATINE-CREATININE METABOLISM AND THE HORMONES

ANTERIOR PITUITARY HORMONES, THEELIN AND TESTOSTERONE PROPIONATE[1,2]

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IT IS GENERALLY believed that a close relationship exists between some of the hormones and the metabolism of the muscles. Most of the studies on this relationship have been concerned with changes in creatine-creatinine metabolism. Korenchevsky (1) reviewed the literature from 1890 to 1925 dealing with the effect of castration and ovariectomy on obesity and on gaseous and nitrogen metabolism. Tsun-Chee-Shen (2), in the only paper published on the subject during this period, reported no effect on creatine metabolism from castration in dogs and in rats. Creatinuria, however, often occurs after castration in rabbits, (3, 4), rats, (5), and dogs (6). It also occurs in some cases of acromegaly (7), pregnancy, and hyperthyroidism (8). Papanicolaou and Falk (9) stated that the general muscular enlargement following treatment of guinea pigs with the anterior pituitary sex hormone offers an explanation for the higher muscular development of the male mammal, and emphasized the importance of obtaining data on the action of the androgenic hormone in conditions associated with muscular deficiency.

The literature since 1925 is confusing, and in view of the importance of the subject, we began some time ago to make a systematic study of the effect of the parenteral injection of various hormones on creatine-creatinine metabolism both in the normal and in the castrated animal. This first report covers the results obtained with an anterior pituitary extract (containing the growth hormone G, the thyrotropic hormone T, and the sex hormone S); antuitrin-S; follutein; testosterone propionate (perandren); and theelin (amniotin).

EXPERIMENTAL METHODS

Young rats weighing between 100 and 150 gm. were placed in cages specially constructed to permit the collection of urine, uncontaminated by food and feces. The stock diet of this laboratory was used (10). The studies were conducted in 2-day periods, as follows.

a. Control period (2 days).
b. Injection of various hormones (8–12 days).
c. Castration (8 days).
d. Second injection of the hormones in castrated animals (8–12 days).

[1] Presented before the thirty-second annual meeting of the American Society of Biological Chemists, Baltimore, Md., April 1, 1938.

[2] Anterior pituitary extract, follutein and amniotin were kindly furnished by E. R. Squibb and Sons, New York; antuitrin-S and T, by Parke, Davis and Company, Detroit; and perandren, by the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

e. Injection of each castrated animal (and also other normal ones) with the hormones for the study of muscle creatine (2 days).

Each animal served as its own control in the urine studies. At 2-day intervals in series *a* through *d*, total creatinine was determined by autoclaving the urine with HCl, followed, as usual, by the use of alkaline picrate; preformed creatinine was determined with alkaline picrate. In series *e* creatine (total creatinine) was determined by the method of Rose, Helmer and Chanutin (11). In a few studies the effect of ether anesthesia and of trauma on creatine-creatinine excretion was also determined.

In the first studies the color comparisons of creatinine in alkaline picrate were made, as usual, by the colorimeter. In the later studies these compari-

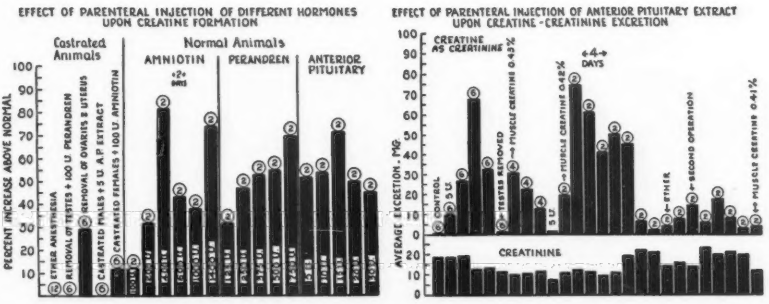

Fig. 1. Effect of parenteral injection of different hormones upon creatine formation.
Fig. 2. Effect of parenteral injection of AP extract upon creatine-creatinine excretion.

sons were made with the Fisher electrophotometer. In most cases the two instruments gave comparable checks. We have shown (12) in several studies that the Jaffe reaction with alkaline picrate is specific for creatinine in muscle filtrates and in urine. This specificity was demonstrated by using the specific creatinine enzyme of Miller and Dubos (13).

RESULTS

Muscle creatine. Figure 1 shows the results obtained following the injection of varying amounts of AP extract (G-T-S), theelin and testosterone propionate. Large and significant increases above normal were observed in muscle creatine in all cases. The results obtained with the AP preparation were especially striking, 5 U being as potent as 30.

Urinary creatine and creatinine. We have observed that the ability of the muscles to hold extra creatine is limited to a short period of time (10). The creatinuria which occurs under these conditions is, therefore, further proof of an increased creatine formation in the body. Figure 2 shows that the injection of AP extract (G-T-S) caused large increases in creatine excretion, which were more marked in the castrated than in the normal animal. Removal of the testes caused significant increases in creatine excretion for about 6 days after the operation. Ether anesthesia and trauma were also followed by a slight increase in creatine excretion. Muscle creatine was not affected,

and there was a slight decrease in preformed creatinine after injection of the hormone in normal and in castrated animals.

The AP preparation which we used, as has already been mentioned, contains the growth, thyrotropic, and sex hormones (G-T-S). It was next

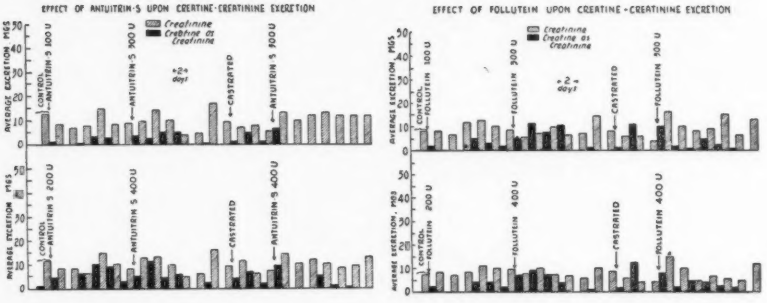

Fig. 3. Effect of antuitrin-S upon creatine-creatinine excretion. Fig. 4. Effect of follutein upon creatine-creatinine excretion.

of interest to study the effect of antuitrin-S and T in this connection. Figure 3 shows the results obtained when antuitrin-S was injected. The increases in creatine excretion were not nearly as striking as those obtained when

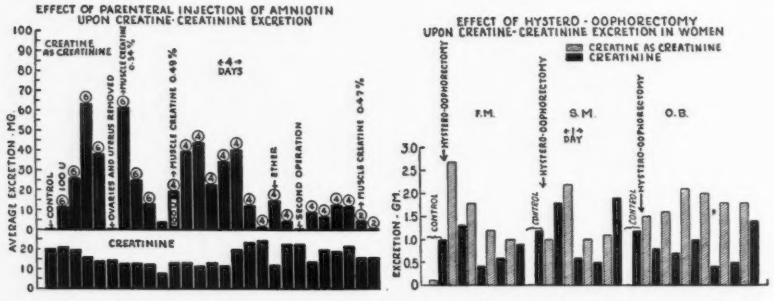

Fig. 5. Effect of parenteral injection of amniotin upon creatine-creatinine excretion. Fig. 6. Effect of hystero-oöphorectomy upon creatine-creatinine excretion in women.

AP extract was injected. In some experiments of Koven and Beard, which are nearing completion at this time, it has been observed that antuitrin-T causes increases in creatine excretion similar to those produced by the use of antuitrin-S. It is possible that the most marked effect of the three AP hormones on creatine excretion may reside in the growth factor, and this may be responsible for the creatinuria which occurs from infancy until puberty in man. Figure 4 shows that the results obtained with follutein, another preparation of the AP sex hormone, are about the same as those obtained with antuitrin-S.

Figure 5 shows that the injection of theelin (amniotin) produced large increases in the total amount of creatine excreted, which were more marked

in the castrated animal. Castration in the female rat caused larger increases in creatine excretion than in the male rat (fig. 5, 2). The muscle creatine was increased and the creatinine excretion was diminished.

It was possible to obtain specimens of urine from 3 female patients in Charity Hospital before and after hystero-oöphorectomy. Figure 6 shows that the very large increases which occurred in creatine excretion were followed in all cases by a large decrease in creatinine excretion. This inverse relationship between creatinine and creatine excretion will be discussed later.

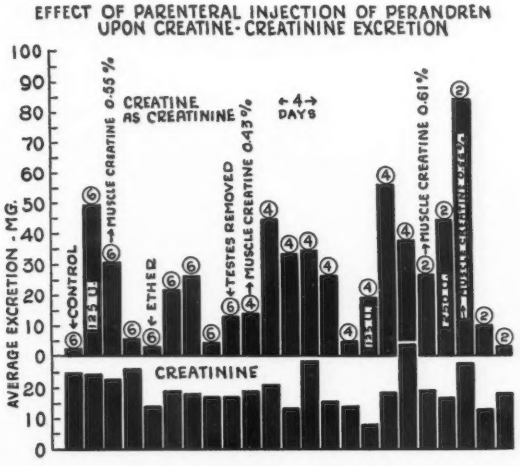

Fig. 7. Effect of parenteral injection of perandren upon creatine-creatinine excretion.

Figure 7 shows the result following the injection of testosterone propionate (perandren). The increases in creatine excretion were about the same as those observed after the injection of AP extract and of theelin, as has just been described, and were much greater, again, in the castrated than in the normal animal. The increases in muscle creatine were very significant. In the first half of the study decreases in creatinine excretion occurred; later, marked variations were observed.

DISCUSSION

The action of the injected hormones on creatine-creatinine metabolism is probably a direct one. This is shown by the fact that histologic changes would probably not take place in the other glands of internal secretion in the 2-day periods following injections. By this we do not mean to imply that one hormone might not affect creatine-creatinine metabolism indirectly through another hormone; we are simply stating that this action was not observed in these studies. Papanicolaou and Falk (9) suggested that the AP sex hormone was concerned in the higher development of the male mammal. Our studies, however, show that the male and female sex hormones, and probably the growth hormone of the anterior pituitary, gave much larger increases in

muscle creatine than antuitrin-S. Thus a definite relation between these hormones and creatine metabolism is established.

The inverse relationship between creatine and creatinine excretion which has already been commented upon has been observed in several studies in this laboratory (12), which show not only that creatinine is transformed into creatine, but also that creatinine injections greatly stimulate both creatine and creatinine excretion as well. On the other hand, we are convinced that creatine is not transformed into creatinine in the body (12). This inverse relationship between creatine and creatinine excretion has been noted by many observers in studies of the creatinurias which occur in such conditions as carbohydrate deficiency, starvation, and the myopathies. We believe that this relationship furnishes irrefutable proof that creatinine is changed into creatine in the body, although this view is not generally accepted at the present time by students of creatine-creatinine metabolism.

The results of the present study are in agreement with those of Nitzescu and Gontzea (14) as to the effect of the anterior growth hormone on creatinuria. They are also in accord with the results reported by Shapiro and Zwarenstein (15) as to the increase in muscle creatine in the South African clawed toad after the injection of this hormone. Osada (16) reported that the oral administration of testis powder to male rabbits and the oral administration of interstitial powder to female rabbits increased the creatine content of the muscle in both sexes, although the creatinine content remained unchanged. Kun and Peczenik (5) reported that creatine disappears from the urine of rats after castration but reappears after the injection of folliculin. They believe that the male hormone regulates the destruction and the female hormone regulates the formation of creatine. Our results, however, do not confirm this view. Most of the hormones so far studied, both by ourselves and by others, increase muscle creatine and creatine excretion.

We are also not in agreement with the conclusions of Schrire and Zwarenstein (17, 18, 19, 20). These investigators observed an increased excretion of creatinine in male rabbits after castration. This did not occur in females. In females the injection of gonadal extracts reduced the high creatinine excretion to the precastration level and the injection of AP extracts caused an increased elimination of creatinine. Zwarenstein studied the creatine excretion in only a few cases. In our experiments on rats the creatinine excretion was diminished. It is of course possible, though not very likely, that some aspects of creatine-creatinine metabolism may differ in the rat and the rabbit. Schrire and Zwarenstein also stated that the creatine-creatinine transformation is controlled by the gonads, but this view is unacceptable to us for two reasons. In the first place, not enough work on the subject has been done to justify such conclusions and in the second place, the transformation of creatine into creatinine does not occur in the rat or in man (12).

At the present time definite results following the injection of various hormones upon creatine-creatinine metabolism have been published by several investigators. From these results it may be concluded that this type of metabolism is closely related to endocrine functions in the body. On the

other hand, it would be unjustifiable to attribute a specific function in this connection to any special hormone. It is more likely that creatine-creatinine metabolism may be controlled by a synergistic action of all the hormones in the body. Further studies in this connection will be reported at a later date.

SUMMARY

Various hormone preparations were injected into normal and castrated rats and the effect of these injections upon creatine-creatinine metabolism was studied.

Amniotin (theelin) 200 to 1400 U; perandren (testosterone propionate) 125 to 750 U; and AP extract 5 to 30 U containing the growth, thyrotropic and sex hormones, caused increases in muscle creatine and in creatine excretion accompanied by decreases in creatinine excretion. Antuitrin-S and T and follutein produced similar but much less striking results. Castration in both sexes resulted in creatinuria, which was more intense in the female and which was accompanied by a decrease in creatinine excretion.

Injection of all of the hormones listed, with the exception of antuitrin-S and T and of follutein, caused a more significant increase in the total amount of extra creatine excreted in castrated animals than in the normal animals. Antuitrin-G is responsible for the creatinuria during early growth in man.

Removal of the testes did not influence the concentration of muscle creatine but removal of the ovaries and uterus caused significant increases in this constituent. Hystero-oöphorectomy in 3 patients was followed for 10 days after the operation by a large creatinuria accompanied by a decrease in creatinine excretion.

This inverse relationship between creatine and creatinine excretion offers irrefutable proof that creatinine is transformed into creatine in the body, and that it stimulates the excretion of both creatine and creatinine in the urine. The transformation of creatine into creatinine does not occur in the rat or in man. A possible synergistic action of the hormones in the body on creatine-creatinine metabolism is suggested.

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PERSISTENCE OF THE 4 TO 5-DAY ACTIVITY CYCLES IN VITAMIN A DEFICIENT RATS WITH CONSTANT CORNIFICATION OF THE VAGINAL EPITHELIUM[1]

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SINCE IT IS WELL known that the spontaneous activity of normal rats has a rhythm paralleling that of the cellular cycle of the vaginal epithelium, it becomes of interest to know whether these activity cycles persist in vitamin A deficient rats whose vaginal epithelium is constantly cornified. Evans (1) reported that vitamin A deficient female rats with constant cornification of the vaginal epithelium accepted the male at regular 5-day intervals, indicating a persistence of the sex rhythm. Moreover, 22 per cent of the copulations resulted in the birth of living young. More recently, Mason and Ellison (2) reported that estrous changes could be detected in the vaginal eipthelium of the vitamin A deficient rat by supravital study of the cells. It was decided then to determine whether the activity cycles persist during the period of continuous cornification and, if so, whether they are altered in any way.

METHODS

Six animals, at an average age of 56 days, were placed in separate activity cages. These cages consisted of a small living compartment, containing a food cup, a graduated inverted water bottle, and a revolving drum with a cyclometer attached. The standard McCollum diet was used. Daily records were made of activity, food and fluid intake, and the animals were weighed weekly. Vaginal smears stained with methylene blue were studied daily.

After the animals had been on the McCollum diet for approximately 15 days, the living compartment was replaced by a larger one containing 3 food cups and 4 graduated inverted bottles. The 3 food cups contained sugar, baker's yeast, and casein (acid washed and autoclaved); the 4 bottles contained olive oil; calcium lactate solution, 2.4 per cent; sodium chloride solution, 3 per cent and tap water. The animals were thus given access to most of the essential dietary constituents except vitamin A (and D), and allowed to make their own selections.

RESULTS

Figure 1 contains 6 records taken from the individual charts kept for each animal. The records begin on the day when the multiple choice diet

[1] Investigation supported by a grant from the Committee for Research in Endocrinology of the National Research Council.

was started and run for periods of 66 to 106 days. The vertical arrows indicate the occurrence and relative height of the peaks of running activity.

It will be seen that for the first 30 to 40 days the cycles of vaginal cornification were quite regular, occurring every 4 days. The activity cycles coincided with the cornification periods. However, with the development of the continuous vaginal cornification, usually about 40 days after the deficient diet was started, the activity cycles continued to occur every 4 days and

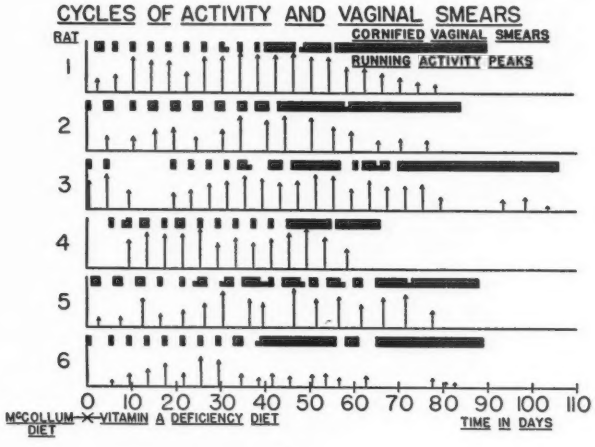

Fig. 1

persisted throughout the period of constant cornification for as long as 50 to 60 days in some cases. The activity usually fell to a low level after about 70 to 100 days on the deficient diet.

DISCUSSION

These results indicate clearly that the cyclical activity peaks, which in the normal female rat correspond in time with the period of cornification of the vaginal epithelium, persist quite regularly for a long time after the continuous cornification due to vitamin A deficiency has set in. The dependence of these activity cycles on ovarian function has been shown (3). There is little doubt, therefore, from this study, and from others in which the condition of the ovaries was periodically examined by laparotomy, that ovarian function continues, even though the vaginal epithelium is constantly cornified. Indeed, Wolbach and Howe (4) have reported that the ovaries of animals in advanced stages of vitamin A deficiency contain ova and follicles in all stages of development up to maturity. The follicular cells show mitoses, and ovulatory function is affirmed by the presence of corpora lutea. It appears, however, that the ovaries are eventually affected by the deficiency, as indicated by the disappearance of the activity cycles and the decrease in daily activity.

SUMMARY

Six rats on a vitamin A-free self-selection diet showed regular activity and estrous cycles for approximately 40 days, each peak of activity coinciding with the appearance of cornified cells in the vaginal smears. After that, the vaginal smears became constantly cornified and no longer showed the regular 4 to 5-day cyclical changes. However, the 4 to 5-day activity cycles still persisted.

The persistence of activity cycles throughout the period of constant cornification indicates that the cycles of the ovary were undisturbed. This is in agreement with results obtained by *a*) laparotomy, *b*) copulation tests, *c*) supravital staining of vaginal epithelium and *d*) postmortem examination of the ovaries.

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SALT TASTE THRESHOLDS OF NORMAL AND ADRENALECTOMIZED RATS[1]

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THE RESULTS of previous self-selection experiments showed that removal of the adrenals in rats greatly increased the appetite for sodium chloride (1, 2). In some instances the appetite as reflected in the voluntary salt intake increased 10 to 18 times over the preoperative level. Incidental observations indicated that adrenalectomy also lowered the taste threshold for salt. With a slightly modified self-selection technic a special study has now been made of salt taste thresholds of normal as well as of adrenalectomized rats.

METHODS

At the outset each rat had access to 2 graduated inverted bottles filled with distilled water. After 8 to 16 days when the intake from each bottle had reached a fairly constant level, one bottle was filled with a sodium chloride solution of an estimated subliminal concentration. Each day thereafter the concentration was increased in small steps until the rats manifested a consistent preference for the salt solution. The concentration of the salt solution at the point of divergence of the water and salt solution intake curves was taken as a measure of the taste threshold.

In order to make certain that any distinction made by the rats between the salt solution and the distilled water depended entirely on the salt content and not on any extraneous factor, the following precautions were observed: the two bottles were cleaned and refilled each day; the distilled water used for the salt solution and for the water bottles was always taken from the same source of supply; the fluids in both bottles were kept at the same temperature; the same two bottles were used throughout the experiment, one bottle for distilled water, the other for salt solution; and each bottle was kept in its original position in the cage.

The rats had constant access to the standard McCollum diet which contains 1 per cent sodium chloride.

In the experiments dealing with the taste threshold of adrenalectomized rats, the operation was performed when the intake from each of the two distilled water bottles had reached a fairly constant level. On the day following adrenalectomy one bottle was filled with the salt solution of estimated subliminal concentration.

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RESULTS

Salt Taste Threshold of Normal Rats

Series 1. In this experiment each rat had access to two bottles of distilled water for a period of 8 days. Then, one bottle was filled with a 0.05 per cent salt solution and thereafter on each successive day the concentration was increased in steps of 0.01 per cent.

During the preliminary period on distilled water the rats drank almost equal and constant amounts from both bottles. On the first day after the salt solution was offered most of the rats drank more salt solution than distilled water. Table 1-A summarizes the results of this experiment on 8 rats. Apparently, the initial concentration of the salt solution in this series was either at or above the taste threshold.

TABLE 1. SALT TASTE THRESHOLD OF NORMAL RATS

A Series 1			B Series 2		
Rat no.	Age at beginning of exper. days	Threshold salt sol. %	Rat no.	Age at beginning of exper. days	Threshold salt sol. %
1	105	0.05	1	102	0.04
2	105	0.05	2	102	0.05
3	105	0.06	3	117	0.05
4	105	0.06	4	102	0.08
5	102	0.05	5	98	0.06
6	102	0.05	6	98	0.07
7	105	0.05	7	92	0.06
8	102	0.07	8	102	0.035
			9	102	0.05
			10	102	0.06
			11	92	0.05
			12	98	0.06
Averages	104	0.055		101	0.0554

Series 2. In this experiment distilled water was offered in both bottles for a period of 14 days. Then one bottle was filled with a salt solution of a concentration of 0.005 per cent, and each day thereafter increased as follows.

1. 0.005 per cent
2. 0.006 "
3. 0.007 "
4. 0.008 "
5. 0.009 "
6. 0.010 "
7. 0.015 "
8. 0.020 "
9. 0.030 "
10. 0.035 "
11. 0.040 per cent
12. 0.045 "
13. 0.050 "
14. 0.060 "
15. 0.070 "
16. 0.080 "
17. 0.090 "
18. 0.100 "
19. 0.200 "
20. 0.300 "

Fig. 1-A gives the average daily intake for 12 normal animals for 5 days when both bottles were filled with distilled water and for 30 days when one

bottle was filled with salt solution. The concentrations of the salt solution are shown in the small graph at the bottom. During the preliminary 5-day period the intake of distilled water from each bottle averaged approximately

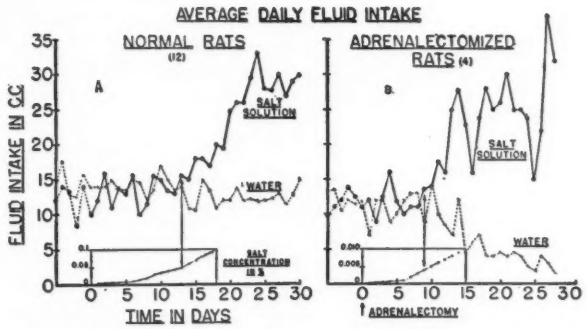

Fig. 1. (A) Daily intake in cc. of water and salt solutions of increasing concentration for 12 normal rats (series 2) and, (B), for 4 adrenalectomized rats (series 3). Threshold concentration is designated by the arrow.

13 cc. per day. After the salt solution was started the intake from the two bottles remained the same until the concentration of the salt solution reached 0.05 per cent. On that day, indicated by the arrow, the intake of salt solution increased slightly. Thereafter it increased fairly steadily reaching a peak of 33 cc. on the 24th day. At this time the daily distilled water intake still averaged 12 cc.

Table 1-B summarizes the results for the 12 rats. The taste threshold

Table 2. Salt taste threshold of adrenalectomized rats

A Series 1			B Series 2			C Series 3		
Rat no.	Age at operat. days	Threshold	Rat no.	Age at operat. days	Threshold	Rat no.	Age at operat. days	Threshold
1	139	0.011	1	136	0.002	1	377	0.004
2	139	0.010	2	139	0.003	2	355	0.003
3	139	0.010	3	122	0.002	3	355	0.004
4	139	0.010	4	136	0.002	4	305	0.004
5	132	0.011						
6	136	0.011						
7	139	0.014						
8	136	0.010						
Averages	137	0.0109		133	0.0023		348	0.00375

averaged 0.0554 per cent and ranged from 0.035 to 0.08 per cent. In summary, normal rats began to distinguish salt from water in concentrations of 1 part of salt to nearly 2,000 parts of water.

Salt Taste Threshold of Adrenalectomized Rats

Series 1. Each animal had access for 16 days to two bottles which contained distilled water. The rats were then adrenalectomized and on the same day one bottle was filled with a 0.010 per cent salt solution. Each day thereafter the concentration was increased in steps of 0.001 per cent. On the first day almost all of the rats drank more of the salt solution than of water. Table 2-A summarizes the results for 8 animals. Just as in Series 1 of normal animals the initial concentration of salt solution was at or above the threshold.

Series 2. In this series the salt solution was started in a concentration of 0.001 per cent on the day of adrenalectomy and increased daily in steps of 0.001 per cent. Here again, on the first day most of the adrenalectomized rats drank more salt solution than water. Apparently the initial concentration was still too close to the threshold. Table 2-B summarizes the results for 4 rats.

Series 3. In these experiments salt solutions were started on the day of adrenalectomy in concentrations of 0.0001 per cent, and increased as follows:

1. 0.0001 per cent
2. 0.0003 "
3. 0.0005 "
4. 0.0007 "
5. 0.0009 "
6. 0.0010 "
7. 0.0020 "
8. 0.0030 "
9. 0.0040 per cent
10. 0.0050 "
11. 0.0060 "
12. 0.0070 "
13. 0.0080 "
14. 0.0090 "
15. 0.0100 "

Fig. 1-B shows the average water and salt solution intake of 4 adrenalectomized animals for a preliminary period of 8 days in which both bottles were filled with distilled water, and for the postoperative period of 27 days in which one bottle was filled with salt solution. The concentrations of the salt solution are shown in the small graph.

In the 8-day preliminary period the intake of distilled water for the two bottles averaged approximately 12 cc. per day. For 8 days after adrenalectomy and after the salt solutions were started the intake of water and salt solution remained equal. When a concentration of 0.004 per cent was reached the rats started to drink more salt solution and less water. On the 27th day the salt solution intake had reached a peak of 38 cc. while the water intake had dropped to 3 cc. per day.

Table 2-C summarizes these results. The salt taste threshold for the 4 animals averaged 0.0037 per cent and showed practically no individual variations at all. In other words, the adrenalectomized rats began to distinguish salt solution from water in concentrations of 1:33,000.

DISCUSSION

These experiments show that normal rats have a definite and consistent taste threshold for sodium chloride. Most of the animals distinguished salt

solution from water in concentrations as low as 1 part of salt to 2,000 parts of water. The taste thresholds were markedly lowered by adrenalectomy. The adrenalectomized animals distinguished salt solution in much smaller concentrations, 1 part of salt to 33,000 parts of water.

In human beings only one report of a lowered salt threshold has come to our attention. Darley and Doan (3) reported a lowered salt taste threshold (0.092 per cent as compared to 0.185 per cent for normals) in a 20-year-old patient who also had a greatly increased salt appetite. She had manifested the signs and symptoms of obstruction of the lesser circulation since childhood and at autopsy marked pulmonary arteriolar sclerosis was found. Unfortunately the adrenals were not studied. However, the changes in appetite and taste threshold for salt strongly suggest some derangement of these glands.

The reason for the preference of normal rats for salt solution is not clear. It may be that the salt content of the McCollum diet does not fulfill all of their salt needs. The great preference for salt solution shown by adrenalectomized animals is clearly dependent in some way on the greatly increased salt need.

The adrenalectomized rats showed a preference for extremely small amounts of salt before having the opportunity to discover the beneficial effects of ingesting large amounts. This indicates that the increased salt intake must have depended on an altered appetite for salt rather than on a trial and error process resulting in relief from insufficiency symptoms or discomfort after ingesting salt. It may be noted also that the amount of salt received in the salt solution at the threshold level, 0.0005 gm. per day, is very small compared to the amount of salt received each day from the McCollum food, approximately 0.1 gm. In adrenalectomized rats the taste receptors of the oral cavity must undergo chemical changes which alter the taste for salt.

SUMMARY

The salt taste threshold of 20 normal rats averaged 0.055 per cent, that is, the animals detected salt in solutions of approximately one part of salt to 2,000 parts of water.

Removal of the adrenals in 8 rats (Series 2 and 3) greatly reduced the salt taste threshold. It averaged 0.003 per cent, that is, the animals distinguished between water and salt solution in a concentration of 1:33,000.

The adrenalectomized rats showed a craving for salt offered in amounts which could not possibly have had any beneficial effect.

The results indicate that adrenalectomized rats ingest more salt, not because they learn that salt relieves their deficiency discomforts, but because of chemical changes in the taste mechanisms in the oral cavity, giving rise to an enhanced salt discrimination.

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THE RENAL LESION IN HYPERPARATHYROIDISM

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AN IMPORTANT advance in knowledge of parathyroid hyperfunction is the fact that lesions of the kidney and parathyroid glands are often closely related. MacCallum drew attention to this relationship in 1905 (1) with his report of a tumor of the parathyroid gland associated with chronic renal disease. Since that time many scattered reports have pointed to some relationship between hyperparathyroidism and kidney disease. Mandl's pioneer work on the surgical treatment of hyperparathyroidism, and his final proof in 1925 of the parathyroid etiology of generalized osteitis fibrosa cystica stimulated much interest in hyperparathyroidism (2). It soon became evident that lesions in the skeletal system were not necessarily the most important, and indeed not even the most frequent pathological change associated with hyperparathyroidism. Due to extensive and careful studies by Albright and his associates (3) in the last few years, the frequency and relatively great importance of renal disease has been recognized.

While numerous cases of parathyroid tumors have been reported, careful histological studies of the kidneys in these cases have rarely been published. Calcium precipitations, either in the form of renal calculi, or as parenchymal deposits, or both, occur with great regularity. From the pathological standpoint, the important problems seem to be whether the calcium deposits are really 'metastatic' or are preceded by degenerative changes in kidney tissue, and whether or not calcium deposition causes damage to kidney tissue to an extent that renal function may be significantly decreased.

Albright suggests that calcium deposition may lead to chronic inflammatory changes with subsequent sclerosis and contraction, simulating chronic nephritis. Chown (4) has suggested that mineral deposits in the kidney will cause changes simulating chronic pyelonephritis. Pappenhemier and Wilens (5) do not agree that calcium deposition will cause permanent kidney damage. The findings in the cases to be reported below indicate that a specific interstitial renal damage occurs in chronic hyperparathyroidism, which is a factor in localization of subsequent calcium deposits. The presence and growth of the calcium deposits causes further reaction and damage, with tubular obstruction and often calculus formation.

Elsom, Wood, and Ravdin (6) noted that in cases of kidney disease associated with a parathyroid adenoma there were certain distinctive clinical features. They state that the common "concomitants of chronic nephritis, e.g. retinal exudates and hemorrhages, cardiac enlargement, and peripheral

edema are absent or very inconspicuous in the reported cases of renal disease and hyperparathyroidism." Despite marked renal failure, increased blood pressure is commonly absent. These distinctive clinical features may be noted in the case reported by Baker and Howard (7), and in the cases reported below. That at least in some cases the kidney damage is not irreversible, is illustrated by Baker and Howard's patient, who showed marked improvement in renal function following removal of the parathyroid adenoma. A second case briefly mentioned by them showed no improvement following operation, but apparently the progression of the kidney damage was stopped. The same appears to be true of the case reported by Bellin and Gershwin (8).

CASE REPORTS

Case 1. A 47-year-old woman came to hospital because of kidney trouble which began 12 years or more previously with bilateral renal colic and the passage of small stones. Six years later trouble again developed with the right kidney, and at that time pyelotomy was performed with removal of 7 small calculi from the pelvis of the kidney. At that time calcification was noted around the cupping of the minor calyces. Following this operation she had occasional attacks of recurrent pain in the kidney region. The last few months before the final admission she suffered from sleepiness, dizziness, headache and visual disturbances. No nocturia or frequency was present. Some edema of the feet and ankles had been noted for a year or more. Some pain was present in the lower extremities.

Physical examination at the time of admission showed temperature 37°; pulse 78; blood pressure, systolic 118, diastolic 74. A small nodule could be felt in the region of the thyroid on the right side of the neck. There was some tenderness over the kidney regions.

Ureteropyelograms showed some hydronephrosis on both right and left sides, but more marked on the right, and some narrowing at the uretero-pelvic junction on each side, with angulation when in the upright position. Calcification of the kidney pyramids was evident in the x-rays. This appeared as encrustations of the calyces with extension up into the kidney parenchyma.

Laboratory test results.

Blood: N.P.N., 22 mg. per 100 cc.; urea nitrogen 14.8 mg. per 100 cc.; calcium, 14.9 mg. per 100 cc.; ionized calcium, 7.4 mg. per 100 cc.; phosphorus, 2.1 mg. per 100 cc.; plasma phosphatase, 11 Kay units; albumin, 4.06 gm. per 100 cc.; globulin, 2.37 gm. per 100 cc.; hemoglobin 12.8 gm.; red blood count 4,220,000 per cu. mm.; white blood count, 8,200 per cu. mm.; polymorphonuclear leucocytes 69%; refractive index, 1.3499; Wassermann reaction, negative; Kahn reaction, negative.

Urine: specific gravity, 1.009 to 1.018; no sugar; albumin, trace; benzidine positive; microscopic, 100 white cells per high power field; Bence-Jones protein, negative.

Kidney Function: Phenolsulphonphthalein excretion, 30 minutes, 20%; 60 minutes, 15%; 90 minutes 10%; 2 hours, 5%; 2 hour total, 50%; appearance time, 5 minutes.

Basal Metabolism: −11 to −17%.

X-ray: Thoracic and lumbar spine showed generalized loss of calcium but with no collapse. Generalized decalcification was evident also in the hands, skull, fibia and fibula. No cysts were evident. The pineal was densely calcified.

Operation was performed with removal of an enlarged parathyroid gland. This was found as a rounded, firm, circumscribed, nodule measuring about 2×1.5×1.8 cm., occupying a position medial to the carotid vessels under the superior pole of the right lobe of the thyroid. This nodule was attached inferiorly by a vascular pedicle to the superior branch of the inferior thyroid artery, and to the thyroid gland by loose areolar tissue.

The results of blood calcium and phosphorus determinations following operation were as follows: 2 hours, calcium 14.1 mg. phosphorus 1.6 mg.; one day, calcium 12.7 mg. phosphorus 1.7 mg.; 2 days, calcium 9.0 mg., phosphorus, 2.7 mg.; 3 days, calcium 9.5 mg. phosphorus 2.9 mg.; 4 days, calcium 9.0 mg.; phosphorus, 2.4 mg.; 5 days, calcium 11.6 mg., phosphorus 2.7 mg.; 8 days, calcium 8.5 mg., phosphorus 3.8 mg.; 45 days, calcium 10.5 mg., phosphorus 3.2 mg.

Six days after the operation she stated that she felt better than she had for years. Five months after the operation she was subjectively well, and had gained 17 pounds. X-rays at this time showed about the same degree of decalcification of the skeleton. Death occurred at home about 6½ months following operation. The kidneys only were made available for postmortem study, and the final cause of death was not determined.

Microscopic sections of the parathyroid tumor showed it to have a dense structure formed by closely packed masses of large, pale oxyphilic cells, and small dark staining chief cells. 'Water-clear' cells appeared entirely absent. The chief cells frequently appeared in small irregular islands set among the more numerous oxyphilic cells. In some areas the oxyphilic cells showed considerable variation in nuclear size, and multinucleated cells occurred. Very little fat could be demonstrated. Dense hyaline connective tissue bands ran through the tissue, and showed some brownish granular pigment. Occasional small islands of parathyroid tissue were enclosed within the bands, separated by the dense hyaline material. Some parts of the tumor were quite vascular, and fresh hemorrhage evident. In one area there was calcification.

The kidneys had very adherent capsules. On the outer surface appeared irregular depressed areas one to two cm. across. In addition, the whole surface was finely granular. On the cut surface there appeared many minute calculi in the parenchyma of the kidney substance, the largest being about 3 mm. in diameter, and concentrated particularly in the apical portions of the pyramids. Several small calculi lay in the calyces of the renal pelvis. The parenchymal tissue appeared scarred and shrunken. A few small, smooth walled cysts, as large as 1 cm. in diameter, were present in the cortical regions. The smaller left kidney weighed 157 gm. and measured 11.5 cm. in length. The pelvis was not thickened or dilated, but showed increased peripelvic fat. The larger right kidney measured 12.5 cm. in length, and had a definitely dilated and thickened pelvis.

Microscopic study of numerous sections from various regions of each kidney showed many interesting features. The most striking finding was the deposition of a granular and amorphous precipitate, the main constituent of which was evidently calcium, as demonstrated by Van Kossa and purpurin strains. This calcium deposition was generalized, but was most massive in the medulla, particularly in the region of the tips of the pyramids. This deposit was mainly interstitial, appearing to involve primarily spaces between and around tubules, the epithelium remaining in most instances intact and uninvolved. In some places the calcium containing mass appeared to have pushed the tubular epithelium ahead of it, producing a bulging into the lumen with narrowing and probably obstruction. Less frequently the tubular epithelium itself appeared involved, sometimes with projection of the areas into the lumen. Actual calcium casts, filling up the lumen of the tubule, without involvement of the lining epithelium, were very rare. The calcium containing masses were in the form of fine amorphous material and as irregular or rounded larger granules. Minute parenchymal calculi in cystic tubules showed some lamination of peripheral portions. In no place was calcification of glomeruli or blood vessels found.

About some of the calcium deposits there were varying but usually small degrees

of monocytic proliferation and fibrosis. In some regions near the pelvis where calcium deposition was marked, striking proliferation of tubular lining epithelium had occurred, becoming 4 or 5 cells in thickness, with marked narrowing of the lumen. About some tubules the calcium formed a sleeve, but not involving the epithelium.

In the cortex, but even more commonly in the medulla, dilated tubules formed thin walled, cyst-like spaces lined by a low cuboidal or flattened epithelium. Some of these contained calculous masses, but others appeared empty, and had bulging into their lumina small calcified papillae covered with epithelium. This appears

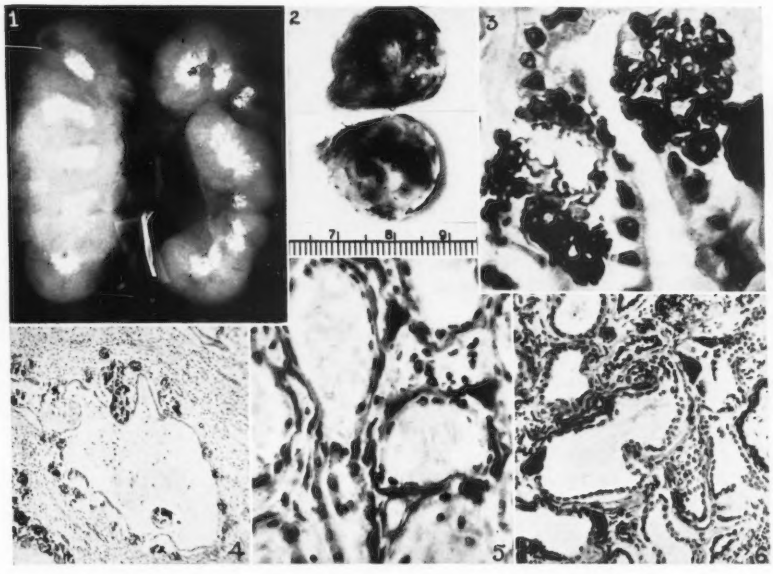

Fig. 1. X-RAY PICTURE OF THE KIDNEYS OF *Case 1*, SHOWING THE LOCATION OF THE MOST MASSIVE DEPOSITS OF CALCIUM. Fig. 2. THE PARATHYROID TUMOR, *Case 1*, AS SEEN FROM THE EXTERNAL AND CUT SURFACE. The dark area seen on the cut surface is a region of massive hemorrhage. The scale is in centimeters. Fig. 3. *Case 1*, PERITUBULAR CALCIUM MASSES DISTORTING LUMEN OF TUBULE, AND ALSO INVOLVING EPITHELIUM. Fig. 4. *Case 1*, DILATED TUBULE IN MEDULLA FORMING A CYST-LIKE SPACE SURROUNDED BY CALCIUM MASSES, SOME OF WHICH ARE BECOMING PINCHED OFF TO FORM TINY CALCULI. Fig. 5. *Case 2*, SHOWS THE THICKENED AND CALCIFIED TUBULAR BASEMENT MEMBRANES, AND SOME PERITUBULAR CALCIUM DEPOSITS. Fig. 6. *Case 3*. CALCIUM DEPOSITION IN TUBULAR BASEMENT MEMBRANES AND PERITUBULAR TISSUES.

similar to the phenomena described by Chown (4) in the kidneys of rats treated by small doses of parathyroid extract over long periods. Generalized tubular dilatation was less marked than described by Chown, though formation of cystic spaces is similar.

Hyalinized and partially hyalinized glomeruli were quite numerous and diffusely distributed. Some showed only varying degrees of thickening of the glomerular capsule. No glomerulitis was present and the majority of glomeruli appeared undamaged. In some areas there was lymphocytic infiltration beneath the pelvic mucosa, and narrow strips of tissue containing interstitial lymphocytic cells radiated to the periphery of the kidney, where there was a slight corresponding depression of the cortical surface. In tissue sections stained for bacteria, organisms were not found.

This case is evidently one in which a moderate degree of hyperparathyroidism had been present for more than 12 years. That it did not become more acute at any time may be due in part to the hemorrhages and fibrosis which occurred in the adenomatous gland, spontaneously decreasing the amount of hyperfunctioning tissue. The massive interstitial calcification in the kidney is in striking contrast to the calcification of acute hyperparathyroidism, which is mainly intratubular, glomerular, and vascular, and only to a slighter extent in the interstitial tissues of the kidney.

Case 2. The clinical aspects of this case have been reported by Hanes (9). The parathyroid tumor was apparently composed almost entirely of small chief cells, and had a fairly loose but highly vascular structure. In some places the tumor cells had a cord-like arrangement. Both recent hemorrhage and old blood pigment were present. Some calcified masses appeared in the microscopic sections.

The kidneys revealed important changes, particularly involving tubular basement membranes and interstitial tissue. Many convoluted tubules showed an irregular thickening of the basement membranes, with degenerative changes and selective calcification limited to the basement membrane or immediately adjacent surrounding tissue. The lining epithelium of convoluted and collecting tubules were involved by varying grades of degenerative changes, and broken up calcified masses partially filled many of these tubules. No such calcium casts were present where the epithelial cells lining the tubules were intact. There was also a massive interstitial calcification similar in all respects to that described in *Case 1*, and a comparable though less marked interstitial fibrosis and cellular infiltration. Hyaline glomerular changes, and particularly tubular dilatation, though similar, were not as prominent as in *Case 1*. In the pyramids irregular calcified masses formed tiny calculi within the kidney tissues.

The striking evidence in this case of connective tissue injury, and particularly of degeneration and involvement of the tubular basement membranes, suggests the mechanism of the injury in chronic hyperparathyroidism. The fairly constant localization of the calcium deposits in a peritubular position as noted in the other two cases reported in this paper, must be a marked deterrent to adequate function of these tubules. This type of calcium deposit should be sharply distinguished from the formation of calcium casts within the lumen of the tubules which hyperparathyroidism has in common with many other conditions having excessive calcium excretion by the kidneys, or selective injury to tubular epithelial cells by certain chemicals.

Case 3. This case has been reported in detail by Howard (10). It seems worthwhile, however, to consider the microscopic findings in the kidneys in comparison with the two other cases. The patient was a male, 19 years of age, with the characteristics of renal dwarfism and a history dating from infancy. While marked renal failure was present, hypertension was conspicuously absent. Autopsy showed generalized parathyroid enlargement, the hyperplasia being mainly of small chief cells.

Microscopic examination of the kidneys showed interesting changes. Most of the glomeruli appeared entirely normal. Some were large and cellular, but evidence of active glomerulitis was surprisingly small. Occasional glomeruli showed hyaline changes and fibrosis. There was no calcification in any of the glomeruli. The outstanding changes were found in the interstitial tissues, adjacent to tubules or Bowman's

capsules. Frequently the calcium deposit formed a sleeve around the tubule. In some places the tubular basement membrane appeared thickened or calcified. The epithelial cells lining the tubules were in good condition, and rarely calcified. In several places the interstitial tissue between dilated tubules has become heavily calcified, with breaking down of the intervening wall and formation of small spaces containing a mass of calcified material. This appears to be the early stage in the formation of small calculi within kidney tissue. A marked increase in the amount of interstitial connective tissue was most prominently seen in the cortex. There was also slight interstitial infiltration of lymphocytes and plasma cells, particularly in the outer layers of the cortex. Many tubules showed marked dilatation. The vascular changes were comparatively slight.

While the opposite possibility must be considered, it seems likely that the parathyroid enlargement seen in this case was secondary to a kidney deficiency. The histology of the parathyroids is similar to the hyperplasias which have been described in association with kidney disease, and unlike that described by Albright as a primary hyperplasia. On the other hand, the interstitial and peritubular calcification with involvement of tubular basement membranes, interstitial fibrosis, and round cell infiltration are entirely comparable to the kidney lesions seen in the cases having a primary adenoma of the parathyroids, and similar to the chronic lesions which have been produced by parathyroid extract in experimental animals.

To ascertain if this type of lesion could be found in kidneys where there was no question of hyperparathyroidism, careful microscopic examination was made of 50 kidneys in which there was calcium deposition. These controls included various types of nephritis, vascular lesions, chemical nephroses, and cases having an excessive intake of calcium salts. In no case was there found a microscopic picture such as has been described in the cases above.

Mitchell (11) in his review of renal rickets, noted that in almost all of the 80 necropsies reported up to 1930 the kidneys showed a chronic interstitial type of involvement. Bader (12) describes in his case of renal rickets a type of chronic interstitial nephritis comparable to those reported here.

From study of the cases described above, it appears that the important effect of chronic hyperparathyroidism on the kidney is involvement of the interstitial tissues. It is probable that there is a specific degenerative effect which precedes the calcium deposit and is a factor in determining its localization.

The probable result of these changes would appear to be interference with tubular function, and extension of these changes as well as the formation of calcium casts result in tubular obstruction. Tubular obstruction produces further glomerular and tubular changes. Any projecting calcium mass froms a nidus on which further precipitation of urinary salts occurs, with production of parenchymal calculi, growing until corresponding glomeruli atrophy. Fibrosis and cellular reaction about interstitial calcium deposits appears to be a minor factor. However, this effect of mineral deposits, by itself, and apart from results of obstruction, deserves further investigation and consideration.

The similarity of the kidney lesions as described in cases of both localized adenomatous overgrowth and generalized hyperplasia of the parathyroids, suggest these glands as an etiological factor in the kidney damage in both cases. It seems possible that in some of these cases the hyperparathryoidism may in turn be due to a primary pituitary disturbance. Chown (4) has already thoroughly discussed the pituitary as the etiological factor in renal dwarfism. Future investigations must determine whether or not the pituitary may initiate a train of events leading to renal lesions.

SUMMARY AND CONCLUSIONS

Chronic hyperparathyroidism is associated with distinctive microscopic changes in the kidney. The characteristic features are interstitial fibrosis, calcification and infiltration by lymphocytes and plasma cells, cystic tubular dilatation, thickening and sometimes calcification of tubular basement membranes, and relative absence of active glomerulitis or involvement of tubular epithelial cells. In individual cases this picture may be varied by calculus formation and superimposed ascending infection. The interstitial calcium deposition is mainly peritubular and is preceded by damage inflicted on kidney tissue, and which would seem to be one of the factors determining the localization of the mineral deposit. Further damage results from interference with tubular function and tubular obstruction. The distinctive clinical features in some of these cases may be related to the relative absence of glomerulitis and vascular changes. The microscopic appearance of the kidney may be distinguished from that of acute hyperparathyroidism as produced experimentally in animals (13).

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THE EXTENT OF REGENERATION OF THE ENUCLEATED ADRENAL GLAND IN THE RAT AS INFLUENCED BY THE AMOUNT OF CAPSULE LEFT AT OPERATION

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THE ADRENAL GLAND of the rat can be enucleated by excising the distal tip of the gland and expressing the body (nucleus) of the remainder of the gland from its capsule. The capsule of the gland and a narrow rim of glomerulosa cells remain attached to the pedicle. When the enucleation is performed on both adrenals or when it is performed on one gland and the opposite adrenal is removed from the body there is a rapid regeneration of cortical tissue from the remaining capsule. For more detailed descriptions of regeneration of enucleated adrenal glands the reader is referred to earlier papers (1, 2). The object of the present study was to determine whether the amount of capsule left at the time of operation may be a factor in determining the extent of regeneration of cortical tissue in the enucleated adrenal gland.

METHODS

All of the animals used in this experiment were male albino rats having an initial body weight ranging from 180 to 190 gm. The adrenal glands were exposed by the usual dorsal approach and the right adrenal was completely removed from each animal. The left adrenal was enucleated by excising varying amounts of its distal tip with small sharp scissors and the nucleus of the remainder of the gland was gently expressed by the aid of fine curved forceps. The nucleus was weighed immediately after delivery since it provided an index as to the area of capsule which remained in the body. During the first 10 days after operation the rats were maintained on a diet which contained 0.6 per cent sodium and approximately 0.3 per cent potassium. The drinking water contained 0.3 per cent sodium. This treatment was adequate to prevent symptoms of adrenal insufficiency after operation. After 10 days the animals were maintained on a regular stock diet which contains 0.24 per cent sodium and 1.0 per cent potassium. The sodium chloride in the drinking water was withdrawn.

Two experiments were conducted. In the first experiment, 100 rats were closely matched into pairs. In one animal of each pair the left adrenal gland was enucleated after removing only a small distal tip from the gland, thus leaving a large amount of the capsule attached to its pedicle. In the other animal of each pair a large portion of the gland was excised thus leaving a

very small amount of capsule after enucleation of its contents. Five pairs of animals were killed at 1 week after operation, 5 pairs at 2 weeks, 5 pairs at 3 weeks, 15 pairs at 4 weeks, 5 pairs at 6 weeks and 15 pairs at 8 weeks.

In the second experiment, enucleation of the left adrenal gland was carried out in 50 rats. Varying amounts of the gland were excised at operation in order to obtain a series of animals in which the amounts of capsule remaining after enucleation were distributed throughout a wide range. All of the animals in the second experiment were killed 8 weeks after operation.

RESULTS

All of the rats gained in weight after operation. After a period of one week no symptoms of adrenal insufficiency were observed. The data on the weights of the regenerating adrenal glands of the animals in the first experi-

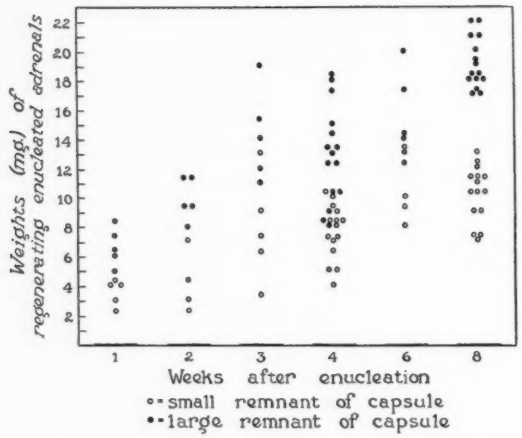

Fig. 1. Weights of regenerating adrenal glands at various periods after enucleation.

ment are summarized in figure 1. It is clearly shown that the weight of the regenerating gland is determined in part at least by the amount of capsule which was left at operation. Larger amounts of regenerated cortical tissue were found among the animals in which large amounts of capsule were left than were found among the animals having small remnants of capsule.

In the second experiment the weight of the nucleus of the adrenal which was expressed from its capsule, after excising the various distal portions of the gland, gave us an index of the size of the capsule remnant which remained after operation. Thus the smaller the weight of the nucleus expressed, the smaller was the remnant of capsule which remained in the animal. A strong positive relationship is shown to exist (fig. 2) between the weights of the nucleus and the weights of the regenerated adrenals 2 months after operation.

COMMENT

The results of this study show that the amount of remaining capsule is one of the factors which determine the extent of regeneration of the enucleated adrenal. These results confirm the results of a study by Ingle, Hales

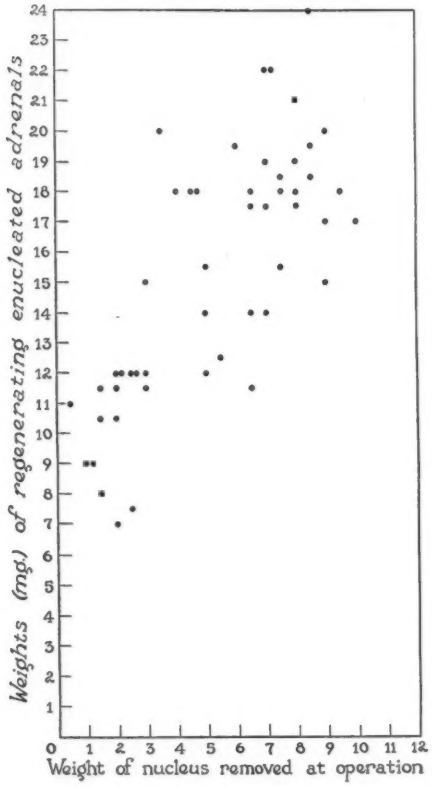
Weights (mg.) of regenerating enucleated adrenals
Weight of nucleus removed at operation
0 1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Fig. 2. Weights of regenerating adrenal glands 2 months after enucleation.

and Haslerud (3), who found that the amount of adrenal tissue in the rat could be limited by partial adrenalectomy.

There are many statements in the literature that either an accessory adrenal or a remnant of an adrenal gland, even microscopic in area, may be capable of increasing in size to that of a normal gland. Our experience does not confirm these opinions. Accessory adrenals or small amounts of adrenal capsule which remain after operation have the capacity for growth and will readily grow to a size adequate to prevent symptoms of adrenal insufficiency but they never gain the size or the functional capacity of the normal adrenal gland.

The method of adrenal enucleation has frequently been described in the literature as a method for adrenalectomy. It is clear that the objectives for which adrenalectomy may be performed can never be attained when even a part of the capsule of the adrenal is allowed to remain in the body.

SUMMARY

In the first experiment the right adrenal gland of 50 pairs of rats was removed from the body. The left adrenal of each rat was enucleated. In one rat of each pair a small amount of adrenal capsule was left intact on the pedicle. In the other rat of each pair a large amount of capsule was left on the pedicle. Pairs of rats were killed at varying periods of time after enucleation of the adrenals and the amounts of regenerated cortical tissue were compared.

In the second experiment the right adrenal was removed from 50 rats. The left adrenal was enucleated, varying amounts of the capsule being left intact on the pedicle. After a period of 2 months the rats were killed. In both experiments a marked relationship was demonstrated between the amount of capsule left at operation and the mass of regenerated cortical tissue.

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THYROID IN THE TREATMENT OF MENSTRUAL IRREGULARITIES

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DURING THE PAST few years we have been impressed by the efficacy of thyroid medication in the treatment of menstrual irregularities. Because our series of cases has shown results similar to those reported by clinicians working elsewhere, it has seemed worth while to summarize our data and present it in confirmation of material published previously (2, 3).

Fifty cases treated with thyroid have been observed for a period of from one to four years. These patients have been seen at least twice a month. Their ages have ranged from 16 to 34, although 41 of the series have been under 22 years of age. All have had complete physical examinations, pelvic studies, and repeated basal metabolic determinations. With a few exceptions, which will be discussed later, only those who showed no pathology in the pelvis have been treated with thyroid.

TABLE 1. SUMMARY OF CASES

Number of cases: 50		
Ages: 16 to 34; 41 were between 16 and 22 years		
B.M.R. range: −1 to −33. Average −15		
Complaints:	Dysmenorrhea	25
	Oligomenorrhea	17
	Amenorrhea	4
	Metrorrhagia	13
	Menorrhagia	7
	Emotional upsets	4

Among this group of patients (table 1) 25 complained of dysmenorrhea which was of sufficient severity to cause repeated absence from classes. Seventeen complained of scanty or delayed menstrual periods with intervals of as long as three months. Thirteen complained of too frequent periods or of inter-menstrual bleeding, while 7 had excessive flow together with the metrorrhagia. There were 5 cases of amenorrhea which was of 7 months to 4 years duration. Four of the patients had normal periods but there was a marked irritability with inability to concentrate for as long as a week prior to the period.

These 50 patients have all had B.M.R. below zero (−1 to −33) the average rate being −15. Blood cholesterol determinations have shown no significant variation from the normal. Each patient has been treated with desiccated thyroid in varying doses, an attempt having been made to raise the B.M.R. to zero or above, although in many cases this was not accomplished because of undesirable subjective symptoms.

The results in this series of cases are summarized in table 2. Of the 25 cases of dysmenorrhea, 17 had complete relief, 5 partial relief, and 3 no relief. Two who had poor results were maladjusted individuals of whom we had the impression that no therapy would be effective. The third patient who was not benefited by thyroid had a third degree retroversion of the uterus. Because she had a moderately low basal metabolism, she was given a therapeutic trial with thyroid before the pelvic pathology was corrected. She later had the uterus put into normal position with complete relief of her symptoms. One of the patients who noted only partial relief had complete freedom from pain until she became involved in some unpleasant social problems which caused her considerable worry and necessitated inadequate rest.

TABLE 2. RESULTS OF THYROID MEDICATION

Complaint	Total cases	Complete relief	Partial relief	No relief	Premenstrual breast engorgement after therapy
Dysmenorrhea	25	17	5	3	
Oligomenorrhea	17	17			
Amenorrhea (8 mo.–4 yr.)	4	1 (8 mo.)	0	3	
Metrorrhagia	13	12	0	1	4
Menorrhagia	7	6	0	1	
Emotional upsets, irritability	4	1	2	1	

A. H., a 17-year-old university freshman, consulted us because of dysmenorrhea which had been present since the onset of her menstrual periods at 14. This was characterized by a cramp-like pain in the lower abdomen the first day of the period, of sufficient severity to necessitate going to bed. Her periods occurred each month until 2 months before seeing us when there was a delay of 1 month. They lasted 4 to 5 days with a moderate flow. There was an additional complaint of early fatigue and a recent inability to concentrate. The physical examination showed a well proportioned white female 66½ in. tall weighing 137 lb. with no abnormalities except a mild hypotension (blood pressure 98/68). The pelvic findings were normal. The results of urine and blood studies were within normal limits. The B.M.R. was −10.

Desiccated thyroid, ½ to 1 gr. daily, was given. The B.M.R. rose to zero; the blood pressure 112/70; there was complete relief of the dysmenorrhea together with an increased ability to concentrate. The patient voluntarily stopped her medication after 6 months of treatment for a period of 9 months. There was a recurrence of the dysmenorrhea and an amenorrhea of 3 months, a gain of 10 lb. in weight, and a fall in the B.M.R. to −9. With thyroid the periods again became normal, the dysmenorrhea subsided and the B.M.R. rose to +1.

E.S., a 22-year-old university senior, consulted the Student Health Department because of severe dysmenorrhea present since the onset of her menstrual periods at 12. There was severe cramp-like pain in the lower abdomen the first day of the period which was so severe she could not attend classes. The periods had always been regular each month and had lasted 5 to 7 days with a moderate flow. Additional complaints were vague abdominal pains, gas and constipation together with an inability to concentrate. This patient was a tall, well developed girl (height 69 in.,

weight 156 lb.) whose physical examination showed no abnormalities. The blood pressure was 98/60. The pelvic findings were normal, and the results of urine and blood studies were normal. The B.M.R. was −14.

Desiccated thyroid, 1 to 2 gr., was given daily. The blood pressure rose to 110/74 and the B.M.R. to +7. There was a complete subsidence of the dysmenorrhea. Also, the abdominal symptoms disappeared and there was an increased alertness so marked that the patient's friends noted it.

Of the 17 cases of oligomenorrhea, all reported definite relief so long as they continued taking thyroid. Several of this group tried going without therapy during the summer vacation but returned in the fall asking to resume treatment because their symptoms had recurred. All stated they felt much better when having their regular periods.

A. T., a university freshman 17 years of age, consulted us because of an amenorrhea of 4 months duration. She stated that her menstrual periods started when she was 11 and that they usually occurred each 6 weeks although there had been two occasions, once when she was 12 and the other when she was 16, when the periods had been delayed 3 months. The flow usually lasted 4 to 5 days and was moderate in amount. There was no dysmenorrhea. The only other complaint was a tendency toward a rapid gain in weight unless she restricted her diet. The physical examination showed a well developed and nourished white female (height 64¼ in., weight 147 lb.) who was apparently normal in every respect with the exception of the blood pressure which was low (92/62) and the moderate obesity. The pelvic findings were normal. The results of urine and blood studies were normal. The B.M.R. was −22.

Desiccated thyroid, ½ to 1 gr., was given daily. The blood pressure rose to 106/80, the B.M.R. to −14; there was the establishment of a 28 to 30-day cycle. The weight remained the same although no restriction in diet was necessary to keep it that way.

E. J., a university sophomore 18 years of age, came to the Student Health Department because of delayed and scanty menstrual periods. Her periods started when she was 12. They had occurred at intervals of 5 weeks to 2 months with a moderate flow lasting 4 to 5 days until 5 months before consultation with us. At that time there was an interval of 2 months followed by a slight flow for one day and then amenorrhea for 3 months. There had never been dysmenorrhea. An additional complaint was a tendency toward rapid gain of weight unless the patient dieted. The physical examination showed a well proportioned female 66½ in. in height weighing 144 pounds, and with no gross physical abnormalities. Blood pressure was 102/68. Pelvic findings were normal. The results of both urine and blood studies were normal. The B.M.R. was −14.

Daily treatment with 1 gr. desiccated thyroid was started. The blood pressure was 102/70; B.M.R. −4; there was a gradual return of the normal flow with monthly periods after 3 months of treatment. Because of the marked improvement, this patient voluntarily stopped her medication. She maintained her improvement for 5 months after this but she had a delayed menstrual period the sixth month so returned for treatment. The B.M.R. was −11. Thyroid again gave normal periods. The B.M.R. was increased to zero at this time.

The group of patients who complained of amenorrhea is small due to the fact that pathology was found to explain the cessation of periods in many of those who had this complaint. Those patients treated were given thyroid

because their B.M.R. were low, and they or their parents wished them to receive a trial with thyroid before endometrial biopsies and further studies were made. One patient had started to menstruate when 13. She had 3 apparently normal periods which were followed by gradual cessation and 4 years amenorrhea. Her B.M.R. was −25. She was given adequate doses of thyroid but with no relief. This was followed by an endometrial biopsy which showed complete inactivity of the endometrium. The one patient in this group who had good results following thyroid medication gave a history of an 8-month amenorrhea. She had a cystic right ovary. Her B.M.R. was −12. She was given a trial with thyroid and after 2 months her periods started. Since that time the menstrual flow has occurred every 28 days although she has severe dysmenorrhea. Pelvic findings have remained the same. It is impossible to say whether thyroid caused the flowing to start. She has been under our observation for a year and has maintained her improvement although no attempt has been made to withdraw her medication.

Those patients complaining of menorrhagia and metrorrhagia responded to thyroid therapy in a most satisfactory manner. The one failure was in a patient who had a large ovarian cyst removed because of prolonged menorrhagia. She had excellent results for a time following surgery, but within a year her symptoms recurred. She had a low B.M.R. and was given thyroid with the hope that she might have some relief, although her remaining ovary was known to be cystic at this time. No relief was obtained but it was felt that this was a case in which thyroid medication could not be expected to give favorable results. Most of the patients in this group have had thyroid medication stopped at times to determine whether they would have a recurrence of symptoms without therapy. Some have maintained their improvement, but most of them have found that the menstrual irregularities recurred.

V. C., a university junior 18 years of age, consulted the Student Health Department because of a slight menstrual flow of six weeks duration, early fatigue, and a tendency toward a rapid gain in weight. She stated that her menstrual periods began when she was 13 and that they had always been irregular, occurring at intervals of three weeks to several months (9 months at one time). The periods usually lasted 5 days although one period had lasted 21 days. There had never been dysmenorrhea. The physical examination revealed a well developed and nourished girl 65 in. tall weighing 131 pounds. There were no abnormalities except a moderately low blood pressure (96/80). There was no demonstrable pelvic pathology. The results of both urine and blood studies were normal. The B.M.R. was −22.

Treatment consisted of 1 to 2 gr. desiccated thyroid daily. The B.M.R. rose to −5, blood pressure to 108/78; the bleeding stopped and apparently normal periods occurred until the dose of thyroid was reduced, when there was a recurrence of the metrorrhagia. This patient has learned that she will have no symptoms if she takes thyroid in sufficient dosage to keep her B.M.R. −5 or above. This has been so consistent in her case that she can accurately predict her B.M.R. will be below these levels when the metrorrhagia recurs.

Among this group of 50 patients, 4 volunteered the information that

they were noting premenstrual engorgement of their breasts, a circumstance which was entirely new to them. All 4 of these had complained of irregularity of periods with an interval of 6 weeks to 3 months, and all had the establishment of regular monthly cycles with thyroid medication.

Four patients who complained of marked irritability prior to their periods are included in this series because their complaint was enough to interfere with their ordinary activities, and 3 did have relief with thyroid. Two noted partial relief, enough so that they did not want to stop thyroid medication. One had complete relief and one noted no difference. Thyroid was stopped several times but always with a recurrence of their symptoms.

W. V., a graduate student 29 years of age, complained of aching in the lower abdomen, inability to concentrate, nervousness and marked fatigue for 10 days preceding each menstrual period. These symptoms were so bothersome that her academic work showed the effects. She stated her menstrual periods started when she was 12 years of age and that they had always been regular each month lasting 5 days with a moderate flow. There was no dysmenorrhea. The physical and pelvic examinations were normal. The results of laboratory studies were all within normal limits except the B.M.R. which was −10.

The treatment was, at first, desiccated thyroid, ½ to 1 gr. daily, followed by a placebo similar in appearance to the thyroid. Considerable subjective improvement was noted, particularly in the irritability. The B.M.R. increased to +2 at this time. For 2 months this patient was given a placebo tablet. She volunteered that the new prescription did not give her the satisfactory results the original had given. Although this patient has not had complete relief with thyroid, her symptoms are always exaggerated when it is stopped.

S. M., a senior student 36 years old, complained of a rather marked emotional instability and depression, inability to concentrate, and fatigue for several days to a week preceding each menstrual period. Her periods began when she was 13 years old and had always been regular each 4 weeks lasting 3 to 5 days. There had been no dysmenorrhea. The results of the physical examination were within normal limits (height 59 in., weight 103 lb.). Pelvic and laboratory studies were normal except for the B.M.R. which was −16.

The treatment was, at first desiccated thyroid, ½ to 2 gr. daily, followed by a placebo similar in appearance to the thyroid. There was a decrease in this patient's subjective symptoms during the administration of thyroid (B.M.R. −4). With the withdrawal of thyroid and the substitution of a placebo her symptoms recurred.

DISCUSSION

The explanation for the efficacy of thyroid medication in these cases can only be hypothesized. Experimental work in animals has shown that ablation of the thyroid is followed by an increase in size of the pituitary gland. Microscopic examination of these pituitaries shows hydropic degeneration, which explains the fact that after this procedure glands lose some of their potency (7). It may follow that in these patients there is an inadequate thyroid secretion resulting in a decrease in potency of the pituitary gland, which in turn causes inadequate gonad stimulation. One might go farther and state that the 4 patients who noted breast engorgement for the first time were having their first normal cycles with luteinization occurring.

Some of these problems may be solved by a study of endometrial biopsies before and after treatment. In a group of patients as young as these reported, most of whom are minors, it is difficult to obtain permission for biopsies from the parents, but as the public becomes educated to this procedure more parents should be willing to consent.

It is our feeling that a B.M.R. should be obtained in all cases complaining of menstrual irregularities such as those reported above. Providing there is no pelvic pathology or physical condition to explain the symptoms, these patients should probably receive a trial with thyroid before further therapy is undertaken if their metabolic rates are below zero. It is understood that these patients will report periodically to their physicians and that the dose of thyroid will depend upon the individual response. In many cases there will be a complete relief of symptoms with small doses of thyroid although the metabolic rate has not reached what is considered the normal level. There is no way of predicting from the individual B.M.R. the degree of relief to be obtained.

SUMMARY

Fifty patients with ages ranging from 16 to 34 years were seen because of dysmenorrhea, oligomenorrhea, amenorrhea, metrorrhagia and menorrhagia. All were apparently healthy individuals with normal pelvic findings. These patients had B.M.R. ranging from −1 to −33 with an average of −15. All were treated with desiccated thyroid. Of 25 cases of dysmenorrhea 17 had complete relief, 5 partial, and 3 no relief. Of 17 individuals with an oligomenorrhea all had complete relief. Of 4 patients with amenorrhea lasting from 8 months to 4 years only one had relief of her symptoms. Among 13 patients with metrorrhagia 12 had complete relief and of 7 with a menorrhagia 6 had complete relief. It is suggested that a B.M.R. should be determined in those individuals complaining of menstrual irregularities who have no demonstrable pathology to explain their symptoms. A therapeutic trial of thyroid is indicated if the B.M.R. is zero or below.

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VITAMIN A DEFICIENCY IN DISEASE OF THE THYROID GLAND: ITS DETECTION BY DARK ADAPTATION[1]

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IT HAS BEEN shown through laboratory studies on animals that certain vitamins are closely associated with the function of the glands of internal secretion. Thus for example: it was observed that animals on an inadequate intake of vitamin B are actually lacking in gonad stimulating hormone of the anterior pituitary gland (1). Considerable experimental evidence has also brought to light the functional relation between vitamin E and the sex hormones (2). It should be stated, however, that in human beings thus far, this relationship has not been sufficiently established. It is not without significance that certain vitamins such as E and D_2 as well as the sex hormones (estrone, testosterone and progesterone) are related structurally to the same cholesterol group. It is apropos of this discussion that some vitamin deficiencies in man may bear certain clinical and physiologic resemblances to endocrine disturbances (3). The coexistence of the two conditions in the same patient is possibly more common than is now being recognized. Greene has recently called attention to the similarity of the gastroenteric, psychic, neurologic, and to a certain extent, dermatologic manifestations of myxedema and pellagra (4). In pursuance of this thought, we investigated patients with various endocrinopathies for vitamin A deficiency by dark adaptation study. Out attention was focused on vitamin A deficiency because some of the manifestations of this condition particularly of the skin, etc., resemble, in a measure, the symptoms commonly observed in hypothyroidism even in the thin individual (5).

There are several laboratory methods for detecting vitamin A deficiency. The present colorimetric method for estimating vitamin A in the blood serum is difficult and exacting and yields results that are too variable to be adopted in clinical work. The spectographic method of analysis holds promise of becoming an accepted quantitative method, but is still in the investigative stage. The dark adaptation method that we selected for our study is accepted by workers in this country to represent the clearest evidence that exists at present for adequacy or inadequacy of vitamin A intake (6). Then again, one of us (7), in a study of over 4000 dark adaptation cases, has found this

[1] Read before The Third International Goiter Conference, Washington, D. C. September 14, 1938.

method to be easily executed and quite accurate for clinical purposes. The method of dark adaptation, to our knowledge, has never before been employed in a study as implied in this presentation.

Dark Adaptation

Dark adaptation is the ability of the eye to adapt itself to darkness. An example of this is the entering into a darkened cinema theatre after walking in the street on a sunny day. At first, it is impossible to see any seats, due to the visual purple being bleached out by the sun. After a specified number of minutes, the objects and people within the theatre appear somewhat clearer. As time goes on, this improvement in vision progresses until the vision becomes as clear as if the theatre were illuminated. This physiologic phenomenon is accomplished through the regeneration of the visual purple in the dark. The regeneration of the visual purple depends, in the main, upon its vitamin A content.

Pathologic dark adaptation is of two types: *a*), organic and *b*), functional. The organic type is a result of deep retinal or choroidal pathology, and is present in diseases such as, retinitis pigmentosa, choroiditis, etc. In the absence of retinal pathology a delay of regeneration of visual purple, above the normal and specified time, is classed as a functional or latent pathologic dark adaptation.

Technique. All tests are carried out in a dark room. The method, as devised by one of us (8), for the study of avitaminosis A consists in uniformly contracting the pupils of all patients examined, to a minimum, by the instillation of pilocarpine or eserine. Thereafter, the study parallels, in the main, the incident of the person walking in the street and then entering the darkened theatre. Each patient is then 'preexposed' to a uniform light of known intensity, for 3 minutes, to bleach out the visual purple. This light is then extinguished and then the light thresholds are taken at 3 to 5 minute intervals; usually for a period of one half hour or more. These light threshold readings, when plotted, are useful for evaluation by comparison with our curves obtained from examining a large number of normal and abnormal individuals.

Material. In this group for study, 59 endocrine patients were examined. They were unselected and taken from the endocrine clinic, the surgical and medical wards of Temple University and Philadelphia General Hospitals. Thirty non-endocrine persons who were examined during this study served as controls.

It was fortunate that among our endocrine patients there were none, at the time of examination, with fever, or such types of liver, gastrointestinal or other conditions known to give a pathologic dark adaptation. Such preliminary laboratory studies as B.M.R. and blood cholesterol determinations, sugar tolerance test and x-ray examination of the sella turcica in pituitary disease, etc., were carried out as was deemed necessary in the individual case. Each patient received a complete physical and ophthalmologic examination. The dietary habits were ascertained with particular reference to the sufficiency of vitamin A intake.

Whenever possible the dark adaptation study was done on the same day as were the other laboratory studies. When necessary, however, as many as 3 or 4 adaptations were taken during the progress of the disease. So that including the control cases, 103 complete dark adaptation studies were made. The list of patients comprised those having disturbances of the pituitary, adrenals and thyroid glands. There were 26 patients in the pituitary and the adrenal series. Our results of the dark adaptation in the series of pituitary and adrenal cases were not at all conclusive to justify an expression of an opinion at this time. The results in the thyroid group were, however, quite consistent. This small group is here reviewed preliminary to our continued study of the subject and with a hope that others may be stimulated to follow up this work.

The group of cases comprised 20 patients with *a*), toxic goiter (including toxic diffuse and toxic nodular goiter. Six patients with *b*), autonomic imbalance, on the borderline of hyperthyroidism, and 7 patients with *c*), hypothyroidism (myxedema and post-operative hypothyroidism).

a) *Toxic goiter.* There were 17 women and 3 men; the age ranged from 21 to 55 years. The B.M.R. varied between +23 per cent to +55 per cent. The duration of the disease, as far as could be ascertained, varied from 3 months to 4 years prior to their admission to the hospital. All had lost weight, but none were in a state of emaciation. The average daily intake of vitamin A in this group exceeded the 4000 I.U. which is considered an adequate daily requirement for adults (9) and as estimated from tables of Eddy and Dalldorf (10). All subsequently had subtotal thyroidectomies, the diagnosis being finally corroborated by microscopic tissue examination. The entire group with the exception of 3 patients had a pathologic dark adaptation and presented a curve noted in latent or functional avitaminosis A, figure 1C. and figure 2B. These 3 patients emphasize a factor, the knowledge of which we found to be essential in the evaluation of dark adaptation; namely, consumption of excessive amounts of food rich in vitamin A (butter, cream, etc.) or prolonged use of iodine prior to study, may vitiate dark adaptation findings.

Two of the three cases gave a high dark adaptation within the normal limit. They gave a history of excessive eating of butter for one month to six weeks respectively, prior to having the dark adaptation studies. It was unfortunate that one of them was operated upon before a dark adaptation recheck could be made. The other patient, however, who was placed in the hospital on a daily intake of 8000 U. of vitamin A had three subsequent examinations taken a number of days apart and on all these tests, the dark adaptation proved pathologic. The third patient with a normal dark adaptation was taking iodine off and on, for a period of 11 months; the last dose of iodine was taken two days prior to the dark adaptation test. No subsequent dark adaptation studies could be done because she was being prepared for thyroidectomy.

We do not wish to draw definite conclusions until a larger series of patients have been investigated. It may, however, be pertinent to integrate our present findings with the experience of several workers in this field. To cite a few of them: St. Rappai and Rosenfeld found that carotene as well as vitamin A lower the B.M.R. in rats receiving thyroxin (11). Carotene likewise prevents weight loss in rats injected with thyroxin (12). According to Eufinger and Gottlieb, the effect of thyroxin in hastening metamorphosis of

tadpoles may be inhibited by the administration of vitamin A (13), and also, the clinical experience of Abelin (14), Wendt (15), Soskin and Mirsky (16), indicating the striking general improvement of hyperthyroid patients treated with diets rich in vitamin A.

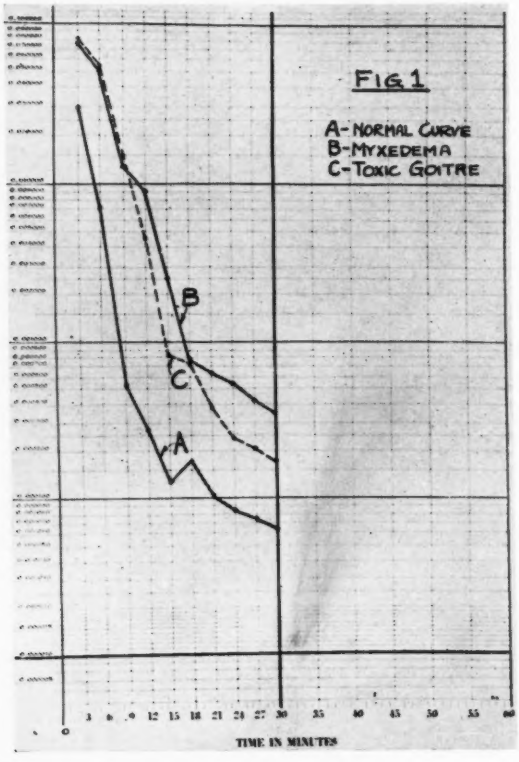

Fig. 1. THREE TYPES OF CURVES OBTAINED FROM ACTUAL CASE EXAMINATION. *A*, normal curve; the peak at the 18 minutes reading denotes inattention on the part of the patient. *B*, advanced pathologic dark adaptation seen in myxedema. *C*, moderately pathologic dark adaptation noted in toxic goiter. The instrument used was the Feldman Adaptometer of The American Optical Company.

We realize that the cases are too few in number, and that some of our patients being prepared for thyroidectomy were receiving Lugol's solution for a limited period varying from a few days to two weeks prior to the dark adaptation test. Iodine may possibly increase the vitamin A value in the body to a greater extent than its capacity to lower the B.M.R. This is suggestive from the fact that in some of our patients the B.M.R. tended towards higher levels than the corresponding dark adaptation readings.

Cases simulating hyperthyroidism. This group consisted of a small (6 cases) but interesting series of patients. They all manifested symptoms of nervousness, tachy-

cardia, and other signs of autonomic imbalance. Three patients were undergoing menopause two of whom had also a goiter; two other patients were young women, 29 and 32 years of age respectively, who had pronounced autonomic imbalance symptoms with loss of weight and some diffuse enlargement of the thyroid. The sixth patient on initial examination presented a persistent tachycardia, loss of weight, B.M.R. +26 per cent and a stimulated mental attitude which mimicked hyperthyroidism. A mitral configuration of the heart with pneumonitis (rheumatic) were disclosed by x-ray examination. A mitral murmur appeared later upon improvement of the patient. The initial B.M.R. in all of these patients was slightly elevated,

TABLE 1. CASES OF HYPERTHYROIDISM SHOWING NO PARALLELISM BETWEEN THE B. M. R. AND THE DARK ADAPTATION CURVES

Name, sex	Age, yr.	B. M. R. %	Dark Adaptation[1]			Diagnosis	Remarks
			Mild	Moderate	Advanced		
.D., M.	36	+19 +23		normal		Diffuse toxic goiter	High vitamin A diet; Lugol's solution 10 min. t.i.d. for 4 wk. prior to test.
M.L., M	24	+64		normal		Diffuse toxic goiter	High vitamin A diet for 4 wk. prior to test 2 subsequent tests were moderately pathologic.
H.H., M	52	+55		pathologic		Diffuse toxic goiter	Lugol's solution 10 min. t.i.d. for 12 days prior to test.
A.M., F	45	+37	pathologic			Toxic nodular goiter	Lugol's solution 15 min. t.i.d. for 14 days prior to test.
L.N., F	56	+40		normal		Diffuse toxic goiter	'Iodine' for 11 mo. and 2 days prior to test.
O.M., F	32	+28	pathologic			Diffuse toxic goiter	Lugol's solution 10 min. t.i.d. 10 days prior to test.

[1] Normal dark adaptation is up to .000150 photons.
Pathologic dark adaptation: mild, from .00015 to .0003 photons; moderate, from .0003 to .0006 photons; advanced, over .0006 photons.

averaging +18.1 per cent (the extremes +14 and +26 per cent). This is in marked contrast to dark adaptations which were all normal. Iodine failed to relieve the symptoms, having no perceptible effect on the basal metabolic rate and pulse rate; further clinical observations finally excluded the existence of thyrotoxicosis.

Our findings appear to bear out the experimental work of Euler (12) and Fasold and Peters (17), that there is a possible antagonism between vitamin A and thyroxin which was evidenced by pathologic dark adaptation in our hyperthyroid patients, and normal dark adaptation in the patients simulating hyperthyroidism. That not insufficient intake of vitamin A was responsible for the pathologic dark adaptation, is apparent from the fact that as a group they consumed large amounts of butter and cream, etc., because of the increased appetite that resulted from the thyrotoxic state. The actual vitamin A intake as estimated from tables by Eddy and Dalldorf exceeded 4000 U in most of them. Then again, two of our patients who were under strict metabolic supervision in the hospital, received a total of 24,000 I.U. of vitamin A,

daily, and on subsequent examinations, the adaptometer readings still continued to be pathologic. This would seem to indicate that thyrotoxicosis rapidly destroys and depletes vitamin A reserves of the body; in what manner it does so needs further elucidation. It is of interest in this connection that Wendt found low carotene values and practically no vitamin A in the blood serum of patients with severe Graves' disease (18).

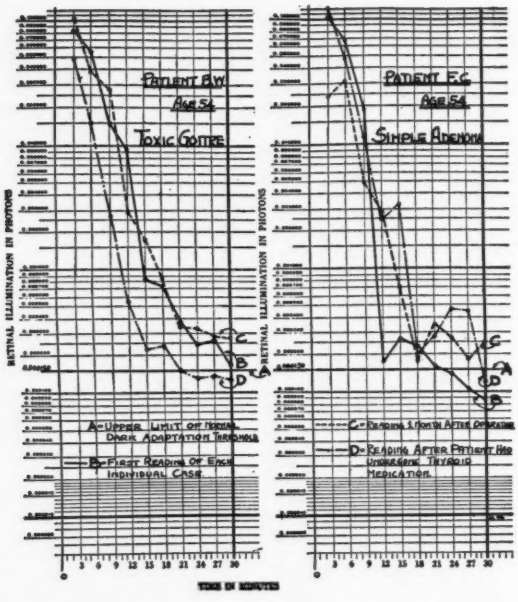

Fig. 2. CHART ILLUSTRATING CHANGES IN DARK ADAPTATION IN TWO PATIENTS OF THE SAME AGE AND UNDER THE SAME DIETARY REGIMEN. Patient B.W., toxic goiter (adenoma), B.M.R. +40%. Patient F.C., non-toxic goiter (simple adenoma), B.M.R. normal. A, Uppermost limit of the normal threshold curve in dark adaptation. B (B.W.), Pathologic dark adaptation noted in toxic goiter. B (F.C.), Normal dark adaptation noted in non-toxic goiter. Both patients underwent thyroidectomy. C, They showed pathologic dark adaptation 3 and 2 months respectively after operation and evidences of hypothyroidism. B.W., B.M.R. −22%; F.C., B.M.R. −16%. Patients placed on thyroid therapy. D, Curves of btho show normal dark adaptation 1 month after treatment. B.W., B.M.R. −10%; F.C., B.M.R. +3%. A Photon is a unit of retinal illumination.

Hypothyroidism. There were 4 patients with myxedema and 3 patients with post-operative hypothyroidism. The B.M.R. varied between −42 to −16 per cent; the blood cholesterol varied between 230 mg. per 100 cc. to 400 mg. per 100 cc. of blood. The degree of vitamin A deficiency as evidenced by dark adaptation and measured by the adaptometer was strikingly pathologic. Figure 1B. gives a representative curve of the group. Their intake of vitamin A in the food was adequate.

The correct interpretation of this apparent discrepancy between the normal vitamin A intake and pathologic dark adaptation can be accounted for by the faulty metabolism and poor assimilation of the vitamin. Two factors might have played a rôle in this group; first lack of complete utilization due to changes in the gastrointestinal mucosa which frequently ac-

companies hypothyroidism hindering proper absorption of vitamin A; secondly a lack of thyroxin.

We are inclined to attribute the marked degree of dark adaptation pathology in our hypothyroid patients to a lack of thyroxin. This contention finds corroboration in the work of Fasold and Heideman (19), who found that the milk in goats which is normally pure white in color, is, after thyroidectomy, yellowish, notwithstanding the fact that the diet is normal. The latter color of the milk is due to a failure of carotene to be converted into vitamin A.

TABLE 2. CASES SIMULATING HYPERTHYROIDISM (BORDERLINE)

Name	Age	Sex	B. M. R. %	Dark Adaptation			Diagnosis:
				Mild	Moderate	Advanced	
A.G.	29	F	+20		normal		Functional neurosis
V.S.	55	F	+18		normal		Menopause with nodular goiter
H.M.	32	F	+16		normal		Neurocirculatory asthenia
M.G.	55	F	+15		normal		Menopause with nodular goiter
C.H.	27	F	+26		normal		Mitral stenosis
A.W	49	F	+14		normal		Menopause

TABLE 3. SIGNIFICANT FINDINGS IN HYPOTHYROIDISM

Name, sex	Age	B.M.R. %	Blood Cholesterol mg. %	Dark Adaptation			Diagnosis
				Mild	Moderate	Advanced	
I.T., M	50	−30	350			Pathologic	Spontaneous myxedema
F.C., F	55	−16	230			Pathologic	Postoperative hypothyroidism
C.D., M	58	−32	300			Pathologic	Spontaneous myxedema
A.F., F	57	−42	380			Pathologic	Myxedema
L.H., F	41	−39	400		Pathologic		Postoperative hypothyroidism
B.W., F	55	−22	275			Pathologic	Postoperative hypothyroidism
H.R., M	10	−22	255			Pathologic	Childhood myxedema

It is interesting, in this connection, to note that Anderson and Soley found carotenemia in 5 patients with clinical and laboratory evidence of hypothyroidism (20). This is in accordance with the work of Wendt who claims that the thyroid hormone is essential for conversion of carotene and also the storage of vitamin A in the liver (21). This, notwithstanding the fact that excess of thyroxin may destroy and deplete vitamin A reserves of the body. Our findings would appear to be consistent with these observations and clinical results of Wendt (22) who reported extremely low values of vitamin A in the blood plasma of patients with cretinism. Furthermore, he observed that the oral administration of carotene in such patients, is not followed by any great increase of vitamin A in the blood.

It is noteworthy that the degree of pathology in all the dark adaptation studies in the hypothyroid group was more marked than in the hyperthyroid cases; so much so, that it was possible to accurately differentiate the hyperthyroid from the myxedema cases just by the definite curve associated with either of the two groups.

SUMMARY

Fifty-nine patients with various endocrinopathies and 30 non-endocrine controls were investigated for vitamin A deficiency by means of the dark adaptation method. Twenty-six cases of this group with pituitary and adrenal disturbances gave variable results. However, the thyroid group gave the most consistent findings. This group included 20 patients with hyperthyroidism, 6 patients with a syndrome simulating hyperthyroidism and 7 patients with hypothyroidism.

It is suggestive from our studies that hyperthyroidism destroys and depletes vitamin A reserves in the body. This is evidenced by the pathologic dark adaptation in 18 of our 20 patients with thyrotoxicosis (90 per cent).

Patients with autonomic imbalance on the borderline of hyperthyroidism (a group of 6 patients) showed normal dark adaptation curves despite an elevated basal metabolic rate. Prolonged clinical and laboratory observation excluded the existence of hyperthyroidism in these patients. One is impressed by the close correlation between the dark adaptation readings and the final diagnosis in these cases.

In the hypothyroid group (7 patients) the dark adaptation was markedly pathologic in every instance; these findings and other data reviewed herein would suggest that the thyroid hormone is essential for conversion of carotene and storage of vitamin A.

The authors wish to express their indebtedness to Dr. W. I. Lillie and his staff for the ophthalmological examinations of these patients.

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THYROID FUNCTION IN DIABETES INSIPIDUS IN THE RAT[1]

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THE PARTICIPATION of the thyroid gland in water metabolism has long been suspected because of the well-known polyuria of hyperthyroidism and the oliguria of hypothyroidism. This has been further related to the hypophysis through the thyrotropic hormone. Barnes, Regan and Bueno (2) showed that the polyuria caused by injections of anterior hypophyseal extracts was prevented by thyroidectomy; the theory followed that the so-called 'diuretic' hormone of the anterior lobe was probably the thyrotropic hormone. This caused diuresis by stimulating the thyroid and hence a hyperthyroid diuresis followed. This phenomenon was, furthermore, related to the diuresis following the placement of a silver clip on the infundibulum of dogs. Mahoney and Sheehan (3) reported this operation to result in a considerable diuresis which could be prevented by a thyroidectomy. Furthermore, since Richter (4) and others have found that total hypophysectomy prevents the diuresis caused by removal of the tissues secreting antidiuretic hormone, these being in association with the pars nervosa, it seemed clear that the anterior lobe's diuretic hormone was customarily working antagonistically to the posterior lobe's antidiuretic hormone.

With further investigation, this simple theory has become much less clear. Biasotti (5) showed that the diureses of anterior lobe administration were concurrent with an increased basal metabolism and were prevented by thyroid ablation, but Gaebler (6) claimed to get some results with such extracts in thyroidectomized dogs. In the rat, evidently neither thyroid nor anterior lobe extracts evoke any diuresis (7, 8). And Barnes, Regan, and Bueno (2) indicate that the pancreas is necessary for the reaction, as well as the thyroid. Furthermore, the theory (3) that the presence of the thyroid is necessary for the diabetes insipidus following inactivation of pituitrin-secreting tissue has been only partially confirmed. Keller (9) agrees with the theory, using dogs, but the Ranson group (10), using cats, report that thyroidectomy does not abolish diabetes insipidus. It does, however, affect it—in some animals slightly but in others considerably, viz., it reduces the polyuria by one half. No measurements of the B.M.R. of these animals were made, but the behavior and appearance of the thyroidectomized cats indicated typical myxedema. Also, when these cats were fed dried thyroid, it caused a return of the large fluid exchange and an exacerbation of existing

[1] A preliminary report has been published (1).

diabetes insipidus. Curiously, even though the thyroid medication was then stopped, the animals maintained the large fluid exchange for 2 to 3 months. White and Heinbecker (11) consider that both the anterior lobe and the thyroid act synergistically to produce diuresis.

That there is considerable species variation in these reactions is shown by White's (8) study on the rat. He has shown, first, that normal rats do not become diuretic under thyroid treatment; second, that thyroid does not exacerbate the diuresis of rats deprived of their posterior lobes (herein called 'posthypophysectomized'); and third, that AP extracts, even when combined with thyroid, do not increase the fluid exchange of either normal or posthypophysectomized rats.

In summary, apparently the thyroid is and yet is not involved. In some species, it appears crucial; in others negligible. Even within the same species, the cat, diabetes insipidus is possible without it; yet it unquestionably aggravates existing diabetes. We determined further to explore its rôle in diabetes insipidus in the rat, by both thyroid administration and thyroid ablation. Also the state of the thyroid's function in diabetic rats was to be determined by measurements of the animals' metabolic rates. The splanchnomegaly of hyperthyroidism and the splanchnomicria of hypothyroidism might also give a clue to the rate of discharge of the animal's own thyroid hormone. Since, also, NaCl has been found (12, 13) to exacerbate the fluid exchange to extreme limits, it was felt that in these exaggerated cases clearer evidence for or against the thyroid's participation in diabetes insipidus might be obtained.

METHODS

The technics for urine collection and measurement of fluid intake have already been described (13). Although both fluid intake and fluid output were followed daily, the records for intake only are herein reported, since the records for output are inaccurate in a small animal like the rat. In the operation making the rats diuretic, the posterior hypophysis, including pars nervosa and pars intermedia, was removed with minimal damage to the anterior lobe. Since in our experience (1) the rat recovers its normal fluid exchange after a period of about 8 weeks, unless it is given saline drinking solutions, either animals which had been recently operated or which had been made very diuretic with saline were employed. It has been suggested (13) that the diuresis of these posthypophysectomized rats given water to drink may differ from that occurring when they are drinking saline solutions. For this reason, the two types of diuresis have been in each case considered separately.

The oxygen consumption was measured after a 12 to 16-hour fast in a simple closed type of apparatus like that reported by Danforth and Loumos (14). At least 4 measurements on 4 different days were taken on each rat; those rats which never gave consistent records were discarded. For acceptance in this study, there were required for each animal at least two records checking within 10 per cent.

Since so many variants are known to affect the rat's metabolic rate (15), a universal 'basal' for the rat is really unknown. Hence our own standards were set up; a group of 55 normal rats showed what might be expected under our conditions and over a range of different body weights. From these data, normal basal metabolism standards were computed. The percentage deviation of the test animals could then be determined. That our method will readily detect physiological differences in oxygen consumption is shown by the fact that thyroidectomized rats averaged a rate of oxygen consumption 37 per cent below normal.

Similar methods have been used to determine whether there were significant changes in the size of liver, kidneys and spleen. A group of 56 normal rats furnished standard weights of these organs beside which the experimental group could be compared.

RESULTS

The oxygen consumptions of the following types of preparation were measured, along with concurrent measurements of fluid exchange.

a), Normal rats on the normal diet. *b*), Rats made diuretic by a posthypophysectomy done two weeks or less before the test (1). *c*), Rats made very

TABLE 1. FLUID INTAKES AND B.M.R. OF DIABETIC RATS

Type of preparation	Number of rats	Average daily fluid intake of group	Average B.M.R. of group
a. Normal	55	9 cc.	0 (standard)
b. Recently posthypophysectomized, drinking water	10	30 cc.	−6%
c. Posthypophysectomized, drinking saline	13	62 cc.	−7%
d. Normal, drinking saline	7	14 cc.	+5%
e. Posthypophysectomized, drinking saline, then thyroidectomized	6	49 cc.	−44%
f. Cretin rats, posthypophysectomized	3	13 cc.	−24%

diuretic from a previous posthypophysectomy combined with the administration of saline drinking solutions (0.4 per cent or 0.9 per cent NaCl). *d*), Normal rats given 0.9 per cent NaCl as drinking solutions, as control of the above. *e*), The same type as *c* above, with an added complete thyroidectomy. *f*), Rats first made cretins by a thyroidectomy and subsequently subjected to posthypophysectomy.

The results are shown in table 1. It is evident from the records of groups b and c that the fluid intake of the operated rats is far above normal—3 times in the case of recently operated animals and 7 times in the case of such animals given saline drinking solutions. But the B.M.R., and therefore presumably thyroid function, are, if anything, slightly depressed in both groups. At all events, the metabolic rates are not elevated as might be expected if a degree of hyperthyroidism were responsible for these diureses. Even extreme diureses were not necessarily accompanied by an elevated oxygen consumption. Two of the rats drinking saline solutions consumed in

fluid daily amounts equal to 89 and 55 per cent of their body weights; their B.M.R. were, respectively, plus 12 and minus 8.

The converse of this condition was also examined: does thyroid ablation prevent these two types of diuresis? The diabetes insipidus of such rats is discussed below, but the table attests the fact that their metabolism was found to be at the low level typical of hypothyroidism.

Another sign of hyperthyroidism is splanchomegaly (16). Hence, liver, kidneys, and heart were weighed in these diuretic rats to see if they were enlarged. Table 2 shows that in both types of diabetic rats, there has certainly been no enlargement in the three viscera but if anything a shrinkage.[2] Evidently, taking this as criterion of thyroid activity, these animals' thyroids are certainly not hyperactive.

The effects of total thyroidectomy on the fluid intake of these diabetic animals were determined in two ways: first, by removal of the thyroid before

TABLE 2. SIZE OF VISCERA IN DIABETIC RATS

Type of preparation	Number of rats	Average daily fluid intake of group	Percentage deviation from normal in weight of		
			Liver	Kidneys	Heart
A. Normal	56	9 cc.	0	0	0
B. Recently posthypophysectomized, drinking water	13	30 cc.	−11	−9	−4
C. Posthypophysectomized, drinking saline	10	42 cc.	−18	−5	−1

the posthypophysectomy and second by removal of the thyroid after posthypophysectomy and salt had established a considerable diuresis.

We had difficulty in preparing cretin rats, but 3 were finally obtained. The operation was done at 25 days of age; after a period of two months, growth had stopped and the B.M.R. averaged −24. The 3 were then posthypophysectomized. In each case the fluid intake was about doubled by the operation, in contrast to an approximate quadrupling caused by the same operation in normal rats (1). Although the group is too small to yield very significant data, it is clear that some diabetes can be caused by a posthypophysectomy in the proved absence of the thyroid. Autopsy confirmed that in all 3 cases the thyroid had been removed.

These 3 animals were subsequently treated with enough thyroid to cause growth increases of 50 gm. in 3 weeks. This did not affect the fluid intake in the slightest—in sharp contrast to the effect of thyroid administration in the thyroidectomized dog or cat.

The effect of thyroidectomy on an existing diabetes insipidus was also studied. The rats were first posthypophysectomized, and then after a period given saline drinking solutions (0.9 or 0.4 per cent) to increase the fluid exchange. They were then subjected to a total thyroidectomy, the completeness of the operation being verified by measurements of the metabolic

[2] The same effect in regard to the liver can be obtained in normal rats with saline. This will be detailed in another paper.

rate. Fluid intakes were measured throughout this sequence. Table 1 shows that their B.M.R. averaged −44 per cent; these were taken 4 weeks after thyroidectomy. Their excretion records were as follows: the daily average intakes of the individual during the 2 weeks before thyroidectomy were, for each of the 2 weeks, 31 and 39 cc. respectively. Daily average intakes of the individual for the 4 weeks following thyroidectomy were, respectively, 27, 32, 37 and 47 cc. The operation reduced the intake for 2 weeks to three-quarters the preoperative intake, but then the group's intake returned to its preoperative level. Even though a temporary reduction was produced by the operation, the animals were still relatively diabetic—that is, they were always drinking at more than 3 times the normal rate.

Pencharz, Hopper and Rynearson (7) and White (8) have shown the rat to be very refractory to AP extracts or thyroid from the point of view of their effects on the water exchange. This refractoriness in the rat we have confirmed in the case of thyroid. Using 4 mg. per kilogram of thyroxin for 3 or 5 days, we followed the fluid exchange of a group of 8 posthypophysectomized and 4 normal rats. Taken as a group, mild diureses of about 20 per cent above normal in both operated and control groups were elicited but this was very inconsistent. Most of the animals, as White noted, showed no effect, but a few showed some response which increased the average of the group. Similarly we have confirmed White's observation (8) of the lack of effect of thyroid extract on the totally hypophysectomized rat. Even, as noted above, thyroid administration to the posthypophysectomized cretin fails to elicit any diuresis.

Up to this point in our experiments, it appears that the thyroid plays only a small rôle in either one of the types of diabetes insipidus, viz. the operative type and the operative type exaggerated with saline. There are two pieces of evidence, however, that implicate it in the condition in the rat: first, morphological and second, experimental. In regard to the morphological evidence, we have noted a degree of enlargement in the posthypophysectomized rats' thyroids. This occurred only after long treatment with saline and resultant long periods of diabetes insipidus. The average weight of the thyroid of 6 of these rats was 38 mg., whereas that of 12 normal rats in the same weight range was 22 mg. The glands then were about twice normal size. Histologically, they were normal in appearance and not hyperplastic as from thyrotropic hormone or (colloid) goitrous as from iodine deficiency. The increase in weight was evidently due to an increase in the number of vesicles. In a few cases of these diabetic rats, considerable thinning and shredding of the colloid was observable but in other cases having a diabetes equal in severity, the colloid appeared normal. We were, then, unable to correlate the degree of diabetes with any structural thyroid change except that of weight.

The second type of observation implicating the thyroid was made in unoperated rats which were made hyperthyroid and then given saline to drink. The intake of water of 5 normal rats over a period of one week averaged 23 cc. per rat per day. (This is higher than previous normal intakes be-

cause the diet was dry; it contained no bread and milk.) The group was then given 0.33 per cent dried thyroid in the diet. During the week, the daily intake averaged 25 cc. per rat—no change from the previous rate. Next the rats' drinking solution was changed to 0.9 per cent NaCl, the thyroid being still in the diet. At once the animal's intakes rose to about double; during the week they averaged 48 cc. per rat per day. During the next 2 weeks the thyroid was removed from the diet, but this made no change in the intakes; they continued to ingest saline at a rate of 50 cc. per rat per day. In another experiment, using the above diet, the previous conclusion that saline administration alone exerts only slight effects on the normal rat's fluid exchange was verified.

DISCUSSION

In the last experiment described, it is clear that neither thyroid alone nor saline alone produced any diabetes in the rats. The combination was necessary for the diabetic effect. But once a degree of diabetes was initiated, it remained whether the stimulating factors were present or not. This recalls the effect that Fisher, Ingram and Ranson (10) report: if a thyroidectomized diabetic cat were treated with thyroid, the fluid exchange remained high long after the thyroid effect had worn off.

The experiment discussed in the paragraph above would, if taken alone, support the hypothesis that the saline effects on the posthypophysectomized rat were due to stimulation of the thyroid and this in turn caused a hyperthyroid diuresis. But all of the other data here reported are clearly opposed to this: as shown, the diabetic rat does not have an elevated B.M.R., it does not have any splanchnomegaly and its thyroids, although enlarged, are not histologically hyperactive.

In order to explain why it is that total hypophysectomy in the rat is followed by only a very transient diabetes insipidus, it has been postulated (4) that the condition is dependent upon 'normal metabolism and general activity.' Fisher, Ingram and Ranson (10) partially agree with this notion. But in this report it has been clearly demonstrated that even if the oxygen consumption is depressed by a thyroidectomy to about half the normal, only slight effects are produced and the animals remain very diabetic. It has already been suggested (13) that the reason why fasting and cachexia reduce a given diabetes insipidus is not that they depress the general metabolism of the animal but rather that they stop the ingestion of NaCl. A total hypophysectomy is also known to depress rats' appetites considerably (17). One wonders whether all the reports of benefit to diabetes insipidus by a thyroidectomy are not due to a resultant anorexia. This question particularly applies to the dog, because in this animal thyroidectomy without injury to the parathyroids is a difficult task (it has, however, been attempted, 3). One of the first and most important symptoms of parathyroid insufficiency is anorexia.

SUMMARY

In a study of the participation of the thyroid in the diabetes insipidus following removal of the posterior hypophysis in the rat and its exacerbation by saline solutions, it has been found that: *a*). The basal metabolic rate and the size of the splanchnic viscera are normal in such rats; hence, presumably, the thyroid is not over-active. *b*). A thyroidectomy previous to posthypophysectomy results in only a light diabetes insipidus, but *c*). A thyroidectomy following saline-exacerbated operative diabetes affects only slightly and transiently the fluid exchange. *d*). A thyroid enlargement occurs in extreme saline diabetes insipidus, but histologically the gland does not appear hyperactive. *e*). Thyroid administration, as others have found, does not influence the rat's fluid exchange, but *f*). If thyroid administration is combined with saline drinking solutions, it causes in the rat an increased and persistent fluid exchange.

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ENDOCRINOLOGIC AND METABOLIC OBSERVATIONS IN EXFOLIATIVE DERMATITIS[1]

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MANY THEORIES have been postulated as to the etiology of chronic exfoliative dermatitis, but none has been substantiated by fact. It is not known whether exfoliative dermatitis is a skin disease or a syndrome of an internal disease; however, it has always been considered in the former classification. Certain skin diseases, such as lupus erythematosus disseminatus and erythema nodosum, which were previously considered as dermatological entities, appear now to be skin manifestations of an internal disease.

Dysfunction of the endocrine glands has long been associated with skin disease. Wolf (1) gives a rather complete summary of the subject. The inflammatory diseases—eczema, erythemas, acne vulgaris as well as the diseases of vasomotor imbalance—urticaria and angioneurotic edema, show cyclic variations during menstruation and pregnancy. Atrophic skin lesions and loss of hair are associated with decreased endocrine activity. Many anomalies of pigmentation occur, for example, with hyperthyroidism and Addison's disease. Psoriasis and scleroderma have been thought by some to be of endocrinologic etiology. It is interesting to note that recently Rosen, et al. (2) have reported chemical changes in the blood in psoriasis. Sutton (3) refers to dermatitis dysmenorrheica which is a dermatosis manifesting itself only during the menstrual period. Myxedema with its characteristic skin findings is definitely the result of thyroid hypofunction. Youmans (4) discusses the change in the skin in thyrotoxicosis—the smooth, thin, elastic, velvety skin with its increased vascularity, heat and moisture, which results in a more rapid shedding of the superficial layers. Busman (5) discusses this relationship aptly in the following comment.

> To establish definitely the etiology of a disease, or group of diseases, to some glandular or metabolic disturbance is usually a very difficult procedure. Cell eruptions are chemical and physical. The chemical agents are applied to the body, introduced into the body and manufactured by the body. The effect of any one of these may be immediate or intermediate, causing cutaneous lesions through a secondary effect on the ductless glands, sympathetic nervous system, digestive system, blood vessels or some combination of these. Thus a skin eruption may be associated with some glandular dystrophy; however both may be the result of a third factor.

Five cases of chronic exfoliative dermatitis are reported in which endocrinologic and metabolic studies were made, perhaps in greater detail than

[1] This paper is based upon an essay by William J. Hanes read before the Medical Staff of the Philadelphia General Hospital, May 15, 1937 and awarded the Internes' Prize for 1937.

Fig. 1. (*Case 1*) *S. L.*, white, age 38: B.M.R. +15 to +55%. Thyroidectomy followed by no effect on skin lesions. Pt. presented amenorrhea associated with hyperthyroidism. Fig. 2 (*Case 1*) Another view of same patient showing exophthalmus. Thyroidectomy scar not visible. Fig. 3 (*Case 2*) *F. S.*, white, age 41; B.M.R. +1 to +40%. This patient showed an increase in B.M.R. and an aggravation of skin lesions with each menstrual period Fig. 8. Note the shortness of stature and the 'pituitary build.' Patient also presented oligomenorrhea. Fig. 4 (*Case 2*) Another view of the same case showing severity of skin lesions which would become red, swollen and exude a serous material with each menstrual period. This also occurred at the time of the expected period when the patient was oligomenorrheic.

in previous articles on the subject. A chance question from one of the patients as to the explanation of the exacerbation of her disease during her menstrual period, with a subsequent subsidence in the intermenstrual period, prompted this investigation. Two female patients and three male patients were studied.

REPORT OF CASES

Case 1. S. L. (fig. 1, 2), a white woman, aged 38. The onset of skin disease was in 1931. This patient presented signs and symptoms of hyperthyroidism, amenorrhea and complained of frequent 'hot flashes.' A thyroidectomy was performed 2 years

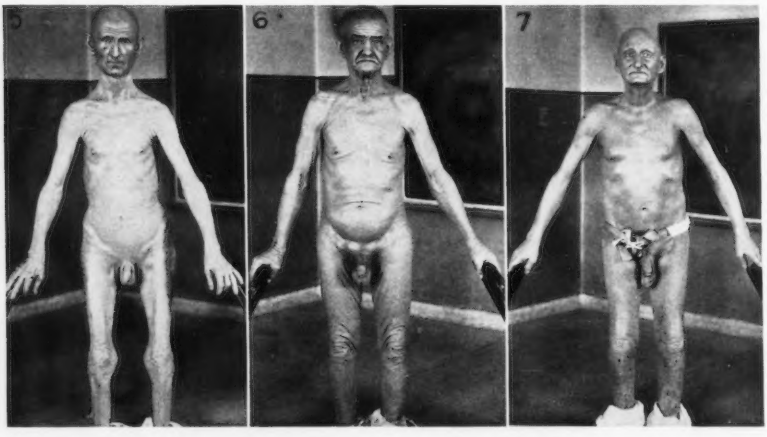

Fig. 5 (*Case 3*) *T. D.*, white, age 58; B.M.R. −1 to +35%. This patient presented impotence and alopecia with the symptoms and signs of hyperthyroidism. Fig. 6 (*Case 4*) *A. R.*, white, age 72; B.M.R. +15 to +36%. This patient presented impotence and alopecia associated with symptoms and signs of hyperthyroidism. This patient showed slight temporary improvement with antuitrin-S therapy. Fig. 7 (*Case 5*) *G. G.*, white, age 58; B.M.R. +27 to +32%. This patient illustrated skin pigmentation, alopecia and impotence as well as signs and symptoms of hyperthyroidism. This patient also showed slight temporary improvement with antuitrin-S therapy.

ago at which time a toxic hyperplastic goiter was found. The B.M.R. was increased; there was decreased blood cholesterol; decreased glucose tolerance and a reversal of the albumin-globulin ratio. She received 900 R.U. of antuitrin and 1800 R.U. of antuitrin-S over a 3-week period with no clinical improvement.

Case 2. F. S. (fig. 3, 4), a white woman, aged 41. The onset of skin disease was in 1927. This patient, unlike the others, did not present the signs and symptoms of hyperthyroidism; however, she did show an increase in B.M.R. and an aggravation of skin lesions with each menstrual period. She did have oligomenorrhea, 'a pituitary build,' and complained of 'hot flashes.' There was a reversal of the albumin-globulin ratio. The results of other laboratory studies were negative, which would be in accord with the fact that this was the mildest case. She received 600 R.U. of antuitrin and 2200 R.U. of antuitrin-S over a period of one month with no clinical improvement.

Case 3. T. D. (fig. 5), a white man, aged 58. The onset of skin disease was in 1934. He presented signs and symptoms of hyperthyroidism, impotence, loss of libido and alopecia. The B.M.R. was raised; the cholesterol was decreased; glucose tolerance

was increased and there was a reversal of the albumin-globulin ratio. He received 1300 R.U. of antuitrin and 3500 R.U. of antuitrin-S over a period of 6 weeks with no clinical improvement.

Case 4. A. R. (fig. 6), a white man, aged 72. The onset of skin disease was in July, 1936. Clinically this patient presented signs and symptoms of hyperthyroidism associated with impotence and alopecia. This man had an elevated B.M.R., decreased blood cholesterol, decreased glucose tolerance and a reversal of the albumin-globulin ratio. Over a 2-month period the patient received 5000 R.U. of antuitrin-S, the result being a slight temporary improvement as noted by decreased exfoliation and lessening of the intense erythema.

Case 5. G. G. (fig. 7), a white man, aged 58. The onset of skin disease was in 1917. Clinically, this patient presented signs and symptoms of hyperthyroidism associated with impotence, alopecia and skin pigmentation. He had an elevated B.M.R., decreased blood cholesterol, a decreased glucose tolerance and a reversal of the albumin-globulin ratio. Over a 2-month period the patient received 5600 R.U. of antuitrin-S, the result being slight temporary improvement as manifest by decreased exfoliation and lessening of the intense erythema.

CLINICAL AND LABORATORY OBSERVATIONS

The female patients (table 1) illustrated menstrual irregularities which showed some relationship to the onset of their skin conditions. One patient

TABLE 1. SYMPTOMS AND SIGNS OF ENDOCRINE DYSFUNCTION IN 5 CASES OF EXFOLIATIVE DERMATITIS

Symptoms or signs of endocrine dysfunction	Case 1 *S. L.*	Case 2 *F. S.*	Case 3 *T. D.*	Case 4 *A. R.*	Case 5 *G. G.*
Impotence			+	+	+
Decrease in semen			+	+	+
Loss of libido	+	+	+	+	+
Frigidity	+	+	+	+	+
Menstrual irregularity	+	+			
Loss of hair	+	+	+	+	+
Signs of hyperthyroidism	+	−	+	+	+
Skin pigmentation	−	−	−	−	+
Shortness of stature	+	+	−	−	−
Hot flashes	+	+	−	−	−
Relation of skin lesion and endocrine system	+	+	+	+	+

(fig. 1, 2) developed oligomenorrhea 2 years previous to the onset of skin disease. With the onset of complete amenorrhea 4 years ago, the skin disease which had previously been local in distribution became generalized. The other patient (fig. 3, 4) has had oligomenorrhea for the past one and a half years. Skin disease began 10 years ago. It was this patient who noted the cyclic exacerbation of skin lesions at the time of her menstrual period, there being increased exudation and erythema at that time. This patient also noted that the longer she was amenorrheic, the more generalized her skin lesion became; the reverse was true in that when menstruation recurred, there was regression of the skin lesions.

Although the female patients had always been undersexed, marked frigidity developed with the onset of the skin disease. The male patients (fig. 5, 6, 7) noted the onset of loss of libido, decrease in semen and impotence at the time the disease made its appearance.

Four of the 5 cases presented signs and symptoms of hyperthyroidism: weight loss, increased appetite, tremor, elevated pulse rate, nervousness, irritability and warm skin. Basal metabolic rate determinations supported these observations and will be discussed later. Although one patient submitted to partial thyroidectomy, in which the biopsy showed a toxic hyperplastic goiter, subsequent to the operation symptoms of hyperthyroidism returned. Removal of the thyroid had no effect of any consequence on the skin lesions.

Both female patients were of very small stature, one being classified as pituitary in type (fig. 3). Both women also complained of frequent 'hot flashes.' Hair loss was noted in all the cases, being more prominent in the males, who became bald, losing their eyebrows, eyelashes, pubic and axillary hair. One patient presented marked pigmentation of the skin.

The B.M.R. determinations (table 2), except for an occasional normal

TABLE 2. B.M.R. STUDIES IN 5 CASES OF EXFOLIATIVE DERMATITIS BEFORE AND AFTER HORMONE THERAPY

	Case 1 *S. L.*	Case 2 *F. S.*	Case 3 *T. D.*	Case 4 *A. R.*	Case 5 *G. G.*
Medication received	Antuitrin and antuitrin-S	(As in case 1)	(As in case 1)	Antuitrin-S alone	(As in case 4)
B.M.R. Before R_I	+15% to +55%	+1% to +40%	−1% to +35%	+15% to +36%	+27% to +32%
B.M.R. After R_I	+12%	+5% to +23%	+10% to +12%	+1%	+49%

reading, were elevated in 4 of the 5 cases, varying from +27 per cent to +55 per cent. Thus the clinical impression of hyperthyroidism was confirmed. In one case (fig. 8) a variation in the B.M.R. determinations occurred coinciding with phases in the menstrual cycle. During the period of oligomenorrhea, this was also observed. During the premenstrual week and menstruation, the B.M.R. was in the vicinity of +40 per cent, whereas in the intermenstrual period it fell as low as −14 per cent on one occasion. Coincident with this elevation in B.M.R., a definite increase in the severity of the skin disease was noticed; likewise, improvement accompanied the decrease in B.M.R.

The blood chemistry findings (table 3) showed the following data: 4 of the 5 patients showed cholesterol concentrations of from 92 to 122 (normal range 100 to 300 with the average 225) and therefore definitely below the usual normal. The relationship of free to esterified cholesterol showed no definite abnormality. Although low cholesterol readings perhaps would not be significant in themselves, associated with high B.M.R., they offer further evidence of hyperthyroidism. All of the patients presented abnormalities of serum albumin-globulin ratio in that the globulin was increased and the

albumin decreased. In 3 a positive Takata Ara test was found. One showed abnormal retention of bromsulphthalein.

Four of the 5 patients presented a decreased glucose tolerance (fig. 9), with the greatest rise in the first hour period. Although this type of curve will occasionally occur in normal individuals, it is also seen in more exag-

TABLE 3. BLOOD CHEMISTRY STUDIES IN 5 CASES OF EXFOLIATIVE DERMATITIS WITH SOME EVIDENCE OF DERANGED LIVER METABOLISM

Blood chemistry studies	Case 1 *S. L.*	Case 2 *F. S.*	Case 3 *T. D.*	Case 4 *A. R.*	Case 5 *G. G.*
Cholesterol Normal 120–300	116	198	92	118	122
Cholesterol esters Normal 60–65%	—	47%	54%	56%	87%
Albumin (normal 3.8–5.3)	4.18	3.10	2.13	2.86	3.15
Globulin (normal 1.6–2.6)	3.51	2.98	2.94	2.87	3.57
Alb./glob. Ratio Normal 1.8	1.19	1.04	.72	.99	.88
Takata Ara test	Pos.	Neg.	Pos.	Neg.	Pos.
Bromsulphthalein test Normal—Up to 6% retention at end of 5 minutes	—	—	1%	8%	2%

gerated form in those patients having hyperthyroidism. The fifth case showed an increased glucose tolerance with a slight rise occurring at the end of the second hour period. This type of curve is often seen in cases of hypopituitarism, but may occur in normal individuals. Therefore one can draw no conclusions from these curves alone in evaluating the data.

The urine hormone assays (table 4) showed nothing unusual. According to the methods here employed, the normal prolan content of urine in women is taken to be 5 to 15 mouse units per liter, and normal estrin content to be 15 to 30 M.U. per liter. Less than 10 M.U. per liter of urine prolan is taken as normal in men. The variations from these normal standards were only slight, suggesting no significant abnormality.

TABLE 4. URINE HORMONE ASSAYS IN 5 CASES OF EXFOLIATIVE DERMATITIS

Normal	Prolan Female 5–15 M. U. per liter	Prolan Male <10 M. U. per liter	Estrin 15–30 M. U. per liter
Case 1. *S. L.*	None	—	20 M. U.
Case 2. *F. S.*	<2 M. U.	—	17 M. U.
Case 3. *T. D.*	—	<8 M. U.	—
Case 4. *A. R.*	—	20 M. U.	—
Case 5. *G. G.*	—	<8 M. U.	—

Incidentally, x-ray of the pituitary region showed slight abnormalities in 4 of the 5 cases studied. One patient exhibited an irregular calcified area above the extremity of the posterior clinoids. In another the sella turcica was found to be more shallow than normal, while the third case showed slight cupping of the floor of the sella. In the last patient the pituitary fossa was somewhat deepened and the floor of the sella and the wings of the sphenoid

were exceedingly thick. No definite pathologic significance can be attached to these findings as they are too often seen in normal individuals.

TREATMENT

From an endocrine viewpoint, prolan deficiency was considered as a possible explanation. Current beliefs concerning the hormonal content in the blood and urine during various phases of the menstrual cycle have been discussed by Wolf (1). Since one of the patients showed slight improvement in skin condition just after the cessation of the menses, at the time prolan is elevated in the blood, it was thought that perhaps prolan was the hormone lacking. Supporting this hypothesis was the fact that certain skin lesions, especially acne vulgaris, are known to improve during pregnancy when prolan in the blood and urine is greatly increased. Further support for this line of reasoning is offered by the work of Lawrence (6) who successfully treated acne vulgaris with antuitrin-S.

The B.M.R. determinations suggested another approach to possible therapy. The study of these determinations in one patient over a 5-month interval (fig. 8) showed a cyclic variation in relation to menstrual periods, grossly exaggerated as compared to that usually observed in normal individuals by Snell et al. (7) and Wakeham (8). It is interesting to note that Starr and Patton (9) produced remissions in hyperthyroidism by the use of pregnancy urine extracts; however their work has not been corroborated.

Since the first hypothesis pointed toward the use of a pituitary hormone as a form of substitution therapy, it was decided to see the effect of this hormone in this series of cases. Because of the relative safety of giving the hormonal products as shown by Hamblen (10), Shoemaker and Rea (11), and Mazer and Katz (12), the trial and error method of employing these hormones was undertaken. The substances used were antuitrin (1 cc.—50 R.U.) and antuitrin-S (1 cc.—100 R.U.) in dosage of 1–2 cc. intramuscularly 3 to 5 times weekly, varying in the individual cases. Patients were observed daily over a 4-month period for effects of the medication on their dermatologic and endocrinologic state.

RESULTS

In those individuals receiving antuitrin and antuitrin-S together, there was a local area of redness, heat and tenderness at the injection site. In one patient there was a definite local exacerbation in the skin lesion. In one patient receiving antuitrin alone, there was a similar reaction; however in the patients receiving antuitrin-S alone, there was no such local reaction. This leads one to believe that antuitrin was locally irritating in these patients, while antuitrin-S was not.

In one female patient, after a week of intensive therapy, premature onset of menses appeared after a period of oligomenorrhea of 2 months (fig. 8). In one male patient there was a slight return of libido following one week's treatment. Basal metabolic rate was reduced in 4 cases and in one case it remained elevated (table 2).

Although no dramatic results were expected, in view of the fact that

all previous therapy had failed on these patients, the effect on the skin condition was rather discouraging, for in 3 patients receiving both products the skin lesion suffered an exacerbation during the treatment, becoming more inflammed and exudative. In 2 cases receiving antuitrin-S alone, there was no such exacerbation noted; in these individuals slight temporary improvement in the form of decreased scaling was noted. The color of the skin due to increased vascularity, which had been reddish-blue, developed a pinkish

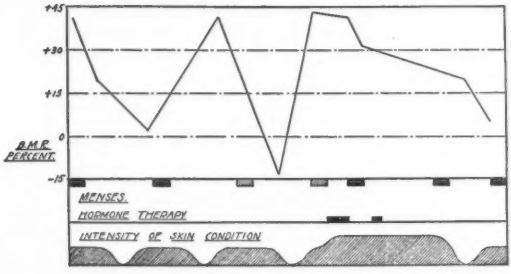

Fig. 8. CLINICAL COURSE OF A CASE OF EXFOLIATIVE DERMATITIS CORRELATING MENSTRUATION WITH THE B.M.R. AND THE INTENSITY OF THE SKIN CONDITION. During the premenstrual and menstrual period, the skin condition was aggravated and the basal metabolic rate was elevated, while in the intermenstrual period, the opposite was the case. The shaded areas indicate the menses which occurred while the thatched areas indicate periods of amenorrhea. Hormone therapy with antuitrin and antuitrin-S precipitated prematurely the patient's menses and aggravated her skin condition.

hue. The manner in which the two hormones affected the skin of these patients, directly or indirectly, again leads one to believe that the endocrine system is in some way involved.

Three patients receiving combined endocrine therapy experienced the sensation of generalized heat over the body for a period of 2 to 4 hours following the injection. Since no such heat reaction occurred following the use of antuitrin-S alone, this was attributed to the antuitrin.

COMMENT

In reviewing this disease from an endocrinologic and metabolic standpoint, the following observations were regarded as significant.

The 2 female patients had menstrual irregularities, oligomenorrhea and amenorrhea, which had made their appearance in relation to the onset of the skin disease. The repeated close relationship in one of these patients between the onset of each menstrual period and an increased intensity of the skin disease (fig. 8) suggests more than coincidence.

The 3 male patients presented impotence, decrease in semen and loss of libido. In reviewing their histories the question might be raised as to whether age might not provide the explanation; however, in 2 of these patients the impotence became manifest concurrently with the onset of the skin diseases at ages of 38 and 55 when they would normally not have lost sexual power.

In considering the alopecia as a possible sign of endocrine dysfunction, it was fully realized that the rubbing of the skin due to pruritus might account for this finding. The skin pigmentation, stature of the patients and the 'hot flashes' that were mentioned were thought to be of only minor significance.

In 4 of the 5 cases there were signs and symptoms resembling those found in hyperthyroidism: namely, weight loss, increased appetite, fine tremors, tachycardia, warm skin, nervousness, and irritability. From a critical viewpoint, one might argue that the warm skin would be present due to the chronic erythema of the skin; and also that with a disease of this nature, any patient would be nervous and irritable. However, associated with the metabolic studies, these findings are suggestive of thyroid dysfunction.

Four of the 5 cases in this series had elevated B.M.R. Each showed also

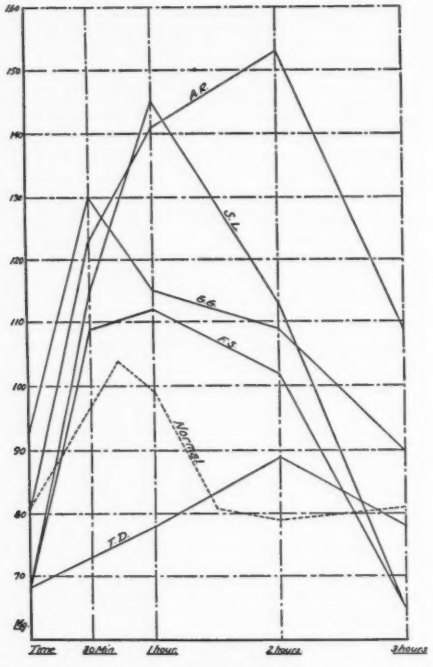

Fig. 9. Glucose tolerance curves in 5 cases of exfoliative dermatitis revealing some abnormalities.

abnormally low serum cholesterol determinations. In considering the significance of these metabolic rates, an interesting question arises—in the presence of a generalized derangement of the skin with an increase in heat loss from the body surface, are the B.M.R. determinations truly indicative of the metabolism of the patient? Although no work has been done which would answer this question, there are two possible explanations. According to one, the B.M.R. is elevated as a compensatory factor because of the augmented heat loss from the patient's skin, thus making the skin disease the primary factor and the thyroid disorder secondary with a compensatory action. While in the other, with a skin disease already present, an elevated B.M.R. associated with thyroid dysfunction secondarily aggravates the

skin. In favor of the former viewpoint, we do know that these patients are constantly complaining of chilliness which points toward excessive heat loss; also, the histopathology of the disease is that of chronic inflammatory tissue with increased vascularity.

Against the former hypothesis and in favor of the latter is the fact that low cholesterols were found in these patients accompanied by glucose tolerance curves which suggest thyroid dysfunction as a primary factor, although the evidence offered by these chemical tests has somewhat doubtful validity because of the probability that chronic liver involvement also existed. A significant clinical observation is the fact that there was an exacerbation of the skin disease in one patient (fig. 8) each time the B.M.R. was elevated. Biopsy of the thyroid of *case 1*, showing a toxic hyperplastic goiter, was another factor supporting the hypothesis that in this case the increased B.M.R. was the primary factor.

The blood chemistry findings also pointed towards a metabolic derangement: the decreased cholesterols, the reversal of the albumin-globulin ratio, the decreased glucose tolerance and the abnormal Takata Ara tests. Such changes are regarded as indicative of liver disease. X-ray of the pituitary region and the hormone assays offered no assistance in establishing the primary agent.

We believe that the present study reveals the existence of dysfunction of the endocrine, hepatic and dermatologic systems in chronic exfoliative dermatitis. At present their relationship to the disease and to each other remains unexplained. It is hoped that some additional evidence will be forthcoming to assist in the evaluation of the factors here indicated.

SUMMARY

Five cases of chronic exfoliative dermatitis are described in which abnormalities suggestive of thyroid and gonadal dysfunction were observed. Evidence was also obtained of abnormality in liver function. The occurrence of variation in B.M.R. determinations during the menstrual cycle was illustrated. Therapy with antuitrin and antuitrin-S showed no conclusive results, although slight temporary improvement was noted in two cases.

The authors wish to express their indebtedness to Dr. John Reinhold for his cooperation in the chemical studies, to the Philadelphia Institute for Medical Research for their aid in the hormone assays and to Dr. E. A. Sharp, Medical Director of the Parke, Davis Research Laboratories, for his liberal supply of hormones for this study.

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THE PRESENT DAY STATUS OF MIGRAINE THERAPY[1]

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FOR THE PAST five years, we have been carrying on research in migraine on the Neurological Service at Bellevue Hospital, under the direction of Dr. Foster Kennedy. During this time, we have treated three hundred patients. The following is a clinical résumé of our therapeutic methods and result.

It is our belief that to satisfactorily treat migraine, one must accept the concept of the conglomerative character of the syndrome. We must treat the condition not as a clinical entity with one cause and one specific cure, but as a clinical symptom that is indicative of some underlying pathological process. This underlying pathological process, whatever it may be, must be determined and treated before one can expect to relieve the suffering of migraine.

Although it is generally admitted that migraine is a syndrome, this concept is neglected by investigators in their therapeutic discussions. The medical literature contains many reports of successful migraine therapy. Each author states, merely, that a certain percentage of these migraine patients was relieved by his method of treatment. If migraine is a symptom caused by many different pathological conditions, one should not expect to find one treatment that is able to cure dissimilar processes. These authors have ignored this concept. For example, the ophthalmologists are confident that the proper fitting of glasses is the correct procedure in giving relief to migraine. Although there are migraine patients whose attacks seem to be precipitated by eye strain, movies, bright lights and prolonged train, bus and automobile trips, there are many other patients in whom these factors have absolutely no relation to the frequency and occurrence of their headaches. The latter patients are disregarded by these authors.

Rhinologists have reported successful results in the treatment of migraine by shrinking membranes and removing septi. Some of the migraine attacks are associated with changes in weather and nasal congestion and are often benefited by such procedures. There are others who are in exceptionally good health. They have fewer colds than the ordinary individual. Their sinuses are unusually clear. They do not know the meaning of a post nasal drip. They have been unaided by previous nasal surgery.

And so on through the medical literature, the allergists, the endocrinol-

[1] Read before The American College of Physicians, New York, 1938.

ogists, the surgeons and the psychiatrists in turn state that the therapeutic solution to the migraine problem is in their hands. They give us no discriminative criteria by which we may select those patients that would be expected to respond to their particular method of treatment.

In order to demonstrate the diverse character of various migraine groups reported in the literature, I have collected several statistical studies for com-

TABLE 1

Group Bib. Reference	A (1)	B (2)	C (3)	D (4)	E (6)	F (7)	G (8)	H (10)	I (11)	J (12)	K (13)	L (14)
No. of Patients	150	55	202	270	60	60	100	137	127	100	12	63
						Percentages						
Females	89	58	67	72	70	71	69	83	75	86	93	76
Onset before age 30	80	82	85	56[1]	[2]	78[3]		84	91		78	91
Family history of migraine	19	45	{82}	57	71	60	39	52	68	89		74
Family history of allergy		85		3	51			29	82			84
Other allergies	89	67	81	3	18		3	41	34	0		51
Food sensitivities	58				78	8		26	37			69
Pos. skin tests		100	100		100							69
Hemicrania	53		94	21		42		86	61			
Aura	39	9	81	76		36		22				5
Nausea	36				{86}	93		93	{100}			85
Vomiting	41					66		76				
Visual					11	36		86		54		39
Precip. factors												
a. emotional	72					35		74	50			61
b. catamenia	17							46	23	94	82	14
c. eyestrain								51	3			13
d. weather								2	8			
Chr. constipation	43					20		30				39
Focal infection	94							49		17		
Hypertension	15			6		10	av.	6		19		
Hypotension	21			10		53	128/80	1		38		
Indigestion						17		39				
Drowsiness			18			11		62				
Polyuria			6					43				
Aphasia			44	{1}				46				
Confusion								10				
Paresthesia			81			{11}		40				
Paresis			6					1				
Gen'l. weakness			40					82				

[1] Before age 16.
[2] Average age 22.
[3] Before age 21.

parison. This analysis demonstrates clearly the discrepancies occurring in the different reported groups. By studying this analysis, one can prophesy the contradictions that do occur in the therapeutic results.

In table 1, are recorded data obtained from 12 separate migraine series. There are noted, in the left hand column, characteristic symptoms which differentiate the migraine attacks from ordinary headaches. These symptoms are: the age of onset, the unilaterality of the headache, the presence of associated gastro-intestinal symptoms, visual phenomena or change of mood during the attack, etc.

In group *A*, only 19 per cent of the patients gave a positive family history of migraine. In group *J*, 89 per cent of the patients stated that other members of the family had suffered from a similar condition. A greater discrepancy exists in the family history of allergies. In series *D*, only 3 per cent of the patients gave a positive family history of hives, hay fever, asthma etc., whereas in groups *B*, *I*, and *L*, over 80 per cent of the patients' relatives had been sufferers of these allergic phenomena.

This wide variation persists throughout the entire study. Seventy-eight per cent of the patients in group *E* gave a history of food sensitivity. In group *F*, only 8 per cent of the patients reacted abnormally to foods ingested. If these physicians were describing a similar pathological condition, one would not expect to find as wide a difference as exists between groups C and *D*, in as characteristic a symptom as the unilaterality of the headache. That the headaches are preceded by an aura, is another cardinal symptom of migraine. Again with this symptom, the variations continue throughout the entire list. It is not surprising that the therapeutic results are contradictory.

Although, these different reports in the literature are contradictory, they are undoubtedly sincere. It is to be expected that the allergist will examine more 'allergic migraine' than the gastroenterologist, and the patients with obvious neurotic stigma will be referred to the psychiatrist. These reports are confusing, however, because we deal with a conglomerative group of migraine sufferers. Our patients are not predominantly of a glandular type or of any one etiologic group. For our purposes, the above reported therapeutic suggestions have proven inadequate.

We have examined 300 patients during the past few years and have used over a dozen substances and methods in a search for a remedy for migraine. In the beginning, we used a trial and error method (5). The first 10 or 20 patients who presented themselves for treatment were given a medication that had been reported as of benefit. To those patients who did not respond to this therapy and to the next 10 patients, who were admitted to the clinic, we gave a second type of treatment, another reported cure or a therapeutic suggestion of our own.

In this manner, we have tried and discarded as of no value in the treatment of migraine many procedures, our own original ideas included. Some of the medications that we used were effective in relieving a fair proportion of cases. We were not satisfied with this trial and error method of prescribing. That there are several different treatments that will relieve some migraine sufferers is not practical. Therefore, we tried to determine what were the symptoms in common presented by those patients who obtained relief from a like drug. By determining these common characteristics, we believed we could establish a symptomatic therapeutic criteria as a guide to therapy.

In order to accomplish this, it was necessary to elicit a very detailed systemic, familial and symptomatic history. As we recorded each symptom on the history chart a code number was given to it, that is headache pre-

cipitated by menses 1; unilateral headache, 10; a personal history of hives, 20; intense vomiting, 84 etc.

These symptom code numbers were arranged across the top of a second chart. According to each patient's symptoms, his initials were placed under his corresponding symptom code numbers. When a patient responded favorably to a particular medication, his initials were charted in color representing the medication that benefited him. In this manner, we have grouped our patients symptomatically and therapeutically on a graph. When a new patient's symptoms are charted and coincide with a particular color group, we prescribe for him the medication indicated. It is by means of this complicated system that we were able to prove many of our impressions. We are now able to break down the complex migraine group into several therapeutic groups.

From the above study, we have observed that emmenin, a placental extract, relieved those women whose headaches are definitely related to the menstrual period, especially if there is a history of scanty or delayed menstruation. It seemed to be of value in relieving the small boned, underweight, Lorain-Levy pituitary type of migraine patient. We have used this hormone either alone or in combination with other medications. It has been prescribed to 101 patients in doses of 2 drams daily, increased to 4 drams daily before the menstrual period (complete data to be reported).

Progynon-B has relieved the headaches of those women who are suffering with concomitant menopausal symptoms. It is also of value in preventing migraine attacks in those women who have had an ovariectomy preceding the onset of migraine, and has been beneficial when administered to those women whose body structure or menstrual history suggests ovarian hypofunction. We have administered this hormone to 124 women; 76 of them obtained relief (to be reported).

Routine allergic studies were done on our patients at the Bellevue and New York University College Clinics by Dr. L. Noun (to be reported). Seventy per cent of our patients gave positive skin reactions. In over 90 per cent of those patients who reacted positively, a history of hives at some time during their lives was elicited. Therefore, those patients who gave a history of hives should be studied allergically by means of skin tests, dietary diaries, diets etc.

It has been our experience that not every patient who reacts positively to the skin tests can be cured by an elimination diet alone. There is often some factor accompanying the atopy which is also an important factor in precipitating the attacks. This pathology must also be determined and treated before cure can be expected.

In those patients with positive skin tests and a history of hives, calcium gluconate and viosterol given daily seemed to diminish the severity and frequency of the attacks. This medication was of particular benefit if the attacks were accompanied by severe nausea and vomiting. It was very effective in lessening these gastro-intestinal symptoms which, to some patients, are more disturbing than the headache.

Each migraine patient should have a basal metabolism determination, especially when the attacks are related to fatigue and strain and are preceded by intense exhaustion. The patients who had basal rates of −20 to −30 have been relieved by thyroid medication alone. Thyroid plus emmenin was satisfactory in those cases who suffered from severe dysmenorrhea and oligomenorrhea with their migraine.

Pituitary extract was beneficial in relieving those migraine patients with large bones, thick noses and thick grimy skins. These 'pituitary headaches' were also associated with the menstrual periods and at times gave allergic stigmata as well.

Any discussion of migraine therapy should not be considered complete without the mention of the use of ergotamine tartrate (9). This drug has not been included in our therapeutic-symptomatic grouping because it is not a cure for migraine. Ergotamine tartrate when injected subcutaneously, in adequate dosage, will terminate the attacks in over 90 per cent of the migrainous patients. Once an attack is aborted, control of future attacks is assured if the drug is given in adequate dosage. We have seen no toxic sequellae from the use of the drug nor have there been any cases of ergot poisoning reported in the literature, from its use in migraine (15). Ergotamine tartrate must be prescribed with care, however. Given orally, the drug is not as effective as when given hypodermically, if 2 or 4 tablets are dissolved under the tongue early in the less severe attacks, they are of benefit in about 69 per cent of the cases.

A therapeutic study, such as the above, reemphasizes the complexity of the migraine syndrome, and the importance of the anamnesis in determining a therapeutic approach to the problem. We believe if one studies the patients with an unbiased mind and treats them allergically, psychiatrically or metabolically as the symptomatology suggests, that this syndrome, though it is complex, need no longer be a therapeutic enigma.

We wish to acknowledge our indebtedness to the Goldman Fund, which has aided in the completion of this work, to the Schering Corporation, to Ayerst, McKenna and Harrison, and to Sandoz for the donations of progynon-B, emmenin and ergotamine tartrate (gynergen), respectively.

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CURRENT ENDOCRINE LITERATURE

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ADVANCE ABSTRACTS

DAVIDSON, C. F. Hypergylycemia without glycosuria associated with disturbances in metabolic proc esses. *To appear in this Journal.*

Case histories, deleted of irrelevant material, are recited as illustrations of instances in which clinical improvement occurred with the application of measures designed to correct hyperglycemia, the existence of which has been demonstrated through glucose tolerance testing. The purpose of the report is to point out the association between hyperglycemia and deviations in a wide variety of metabolic processes and to advance the theory that hyperglycemia may be one of the determining factors in certain clinical manifestations.

FEVOLD, H. L., V. M. FISKE AND I. T. NATHANSON. Physiological reactions produced by gonadotropic hormone from the urine of a case of chorio-epithelioma. *To appear in this Journal.*

The gonadotropic substance excreted in a case of chorio-epithelioma, when injected for 5 days increases the weights of the ovaries of immature rats 14-fold, ovaries of pigeons 7-fold, testes of pigeons 8-fold and produced marked follicular and lutein development in the ovaries of immature hypophysectomized rats when given in large doses, (above 2000 U). Further evidence is presented showing this substance to be the same as the gonadotropic hormone found in pregnancy urine.

FRIEDMAN, M. H. AND G. S. FRIEDMAN. Seasonal variation in the gonadotropic hormone content of the rabbit pituitary. *To appear in this Journal.*

The gonadotropic hormone content of the female rabbit pituitary is rapidly depleted in the 24 hours following sterile coitus. For the first 6 days of the ensuing pseudopregnancy there is not much progress in restitution of the hormone content, the glands remaining almost as low as the 24-hour post-coitum glands. Between the 6th and 10th day of pseudopregnancy, however, the gland content rises rapidly so that the initial estrous level is recovered. Repeated assays of glands from estrous rabbits, and from females in each stage of pseudopregnancy over a period of 2 years has revealed a regular pattern of variation for all types of glands. In general glands obtained in the winter have shown the lowest hormone content, and those obtained in the spring, the highest content.

HIMWICH, H. E., S. J. MARTIN, F. A. D. ALEXANDER AND J. F. FAZEKAS. Electrocardiographic changes during hypoglycemia and anoxemia. *To appear in this Journal.*

The activity of the autonomic nervous system of dogs was studied during hypoglycemia and anoxemia. Electrocardiographic changes showed that in both conditions, an initial transitory sympathetic influence was followed by parasympathetic predominance. This increased activity of the autonomic nervous system was associated with a depressed metabolism of the brain as indicated by diminished cerebral utilization of oxygen. It is concluded that with depression of the brain metabolism, the predominant and ultimate effect on the autonomic nervous system is a release of parasympathetic influence.

JONES, M. S. A study of thyrotropic hormone in clinical states. *To appear in this Journal.*

Several methods for the extraction of pituitary thyrotropic hormone added to normal urine are described; acetone and alcohol-ether methods gave a very satisfactory recovery, as judged by the response of the chick thyroid. Urines from 2 cases of myxedema, 1 case of post-operative hypothyroidism, and 2 acromegliacs were tested for thyrotropic activity; 1 case of myxedema gave results suggesting possible thyrotropic activity. Sera from 9 schizophrenic patients did not show any anti-thyrotropic activity when injected into chicks along with known amounts of thyrotropic substance.

KOTZ, J., AND E. PARKER. The clinical interpretation of the endometrial biopsy, report of three hundred cases. *To appear in this Journal.*

Endometrial biopsies from completely controlled cases in which ovarian dysfunction was suspected have been studied and classified. Abnormalities, which occurred in every stage of development, have been described and interpreted in terms of disturbance of the functional level of the ovarian hormones.

SURE, B., AND R. M. THEIS. Influence of hyperthyroidism on vitamin C content of various endocrines and tissues. *To appear in this Journal.*

Two factors were found responsible for tremendous reductions of ascorbic acid in endocrine glands and tissues: *a*), vitamin B_1 deficiency, and *b*), thyrotoxicosis. Administrations of vitamins B_1 and C counteracted to a large extent such ascorbic acid losses in the body of the rat. The fact that one vitamin (B_1) deficiency can influence marked reduction of another vitamin (C) establishes definitely a vitamin interrelationship.

THOMPSON, K. W. The termination of pregnancy of dogs by gonadotropic antihormone. *To appear in this Journal.*

The interruption of 12 pregnancies in dogs has been accomplished by the intravenous injection of a non-species-specific anti-gonadotropic canine serum. The action of the antiserum was comparable to a physiological hypophysectomy, and it may have occurred by conferring on the injected dog a passive resistance (immunity) to her own pituitary gonadotropic hormone. Normal canine serum did not produce this effect. The antihormone did not cause any objective permanent ill effects in 3 aborted dogs, 2 of which later became pregnant and were again aborted, and one which had a period of estrus. The aborted fetuses which were observed at delivery were alive, but subsequently died of prematurity and lack of maternal care. The antihormone preparation used had no appreciable oxytocic acitivity when tested on the guinea pig uterus, but it contained a considerable amount of antidiuretic hormone, in addition to thyrotropic and gonadotropic antihormones.

WHITE, W., D. A. MCGINTY, L. P. ANDERSON AND F. R. WHITE. The influence of desiccated thyroid, thyroid concentrate, and thyroxine on the oxygen consumption of the guinea pig. *To appear in this Journal.*

A thyroid concentrate prepared in accordance with the published method of Blum was compared with desiccated thyroid and thyroxine in its ability to increase the oxygen consumption of the guinea pig. Blum's concentrate has approximately the same effect as dessicated thyroid in raising the basal metabolism when given in the same equivalents of iodine. No evidence was obtained which would indicate that Blum's concentrate is superior to desiccated thyroid in increasing the oxygen consumption.

GONADS

HAMILTON, J. B., AND S. L. LEONARD. The effect of male hormonal substance upon the testes and upon spermatogenesis. *Anat. Rec.* 71: 105. 1938.

Forty-one rats of the same age and inbred strain were hypophysectomized, and in each rat one testis was confined by ligature in the scrotum, the other in the abdomen. Half the group received injections of testosterone acetate or propionate; the other half of the group served as controls. Injected and comparable control animals were killed at intervals from 1 to 44 days after hypophysectomy. Other rats were injected with testosterone acetate or propionate from weaning until they were 4 mo. old. The following conclusions are suggested with regard to the rat. These testosterone compounds maintain spermatogenesis in scrotal testes of hypophysectomized rats, but do not stimulate spermatogenesis *a*), precociously or *b*), after posthypophysectomy degeneration has ensued. Testosterone acetate and propionate affect tubular tissue, not only when the testis is in the scrotum but also when the testis is in the abdomen. In comparison with control animals the effect on cryptorchid testes consists of a greater testicular weight, larger tubules and a more rapid degeneration of tubular contents. This effect on cryptorchid testes indicates that androgens have their effect not only indirectly by scrotal normality and placement of the testes within the scrotum, but also directly upon tubular tissue. Discussion is given of the possible mechanism of this effect on cryptorchid testes and of the usefulness and limitations of androgens in the treatment of sterility.—Author's Abstract, Wistar Bib. Service.

HAWKINSON, L. F. The menopausal syndrome: Treatment with estrogen. *J.A.M.A.* 111: 390. 1938.

The results in the author's series of 1000 consecutive patients presenting menopausal symptoms treated with estrogenic substances show that 691 were relieved of the majority of all symptoms, 149 were improved, 109 were doubtful and 51 obtained no relief. Results were evaluated by the disappearance of symptoms and by changes in the vaginal smears. The relief of symptoms is usually gradual. Flushes and chills, excitability, irritability, depression and crying, palpitation and insomnia usually disappeared after the seventh or eighth injection of 10,000 I.U. of estrogen in oil or after from 2 to 3 weeks of adequate oral

treatment. Sweating, fatigue, lassitude and headaches responded after further administration of estrogenic preparations. Occipitocervical aching, a symptom complained of by 403 patients, proved very amenable to therapy. Migraine is often completely relieved by adequate estrogenic therapy. The administration of estrogen had little effect on the obesity that was present in some patients. So-called menopausal arthritis seldom responds to estrogenic preparations. It is doubtful whether it should be included as a menopausal condition. If treatment is withdrawn after initial relief, symptoms are almost certain to recur within 2 to 6 weeks. This stresses the importance of continuous oral therapy with a gradual reduction of dosage until the patient is able to discontinue therapy and remain symptom free. Menopausal symptoms are often persistently troublesome and the average patient must remain on maintenance doses of estrogen for from 2 to 3 years. Patients at the menopause who are still menstruating may be more difficult to control, owing to the fact that symptoms are frequently intensified about a week prior to the menstrual period. Also, excessive bleeding may become a problem in these women, and large doses of estrogen may increase the already profuse flow. Oral therapy in the form of emmenin is best suited to these cases. No ill effects were seen in any patient even when doses up to 100,000 I.U. per week were given over a period of many weeks.—Abstract service *J.A.M.A.*

JOHNSTON, J. A., H. A. HUNSCHER, F. C. HUMMEL AND M. F. BATES. The basal metabolism in pregnancy. *J. Nutrition* 15: 513. 1938.

The basal metabolism and the mineral balance were determined concurrently on a young primipara for 66 days ante-partum and 62 days postpartum. The rise in metabolism throughout the latter part of pregnancy does parallel strikingly the cumulative nitrogen and sulphur curves, and this fact constitutes an argument in favor of the concept that the increase is merely the result of increased protoplasmic mass. Since this concept must ignore a number of well-established glandular changes in pregnancy, or make the assumption that these have occurred without influencing the basal metabolism, and since the rise in metabolism is accompanied by a relative fall in the percentage of calories derived from protein, it is felt that a hormone influence responsible for the synthesis of a new mass of protoplasm must likewise be included in any explanation of the phenomenon that seemed complete.—Author's Abstract, Wistar Bib. Service.

JONES, H. W., AND P. G. WEIL. Corpus luteum hormone in early pregnancy. *J.A.M.A.*, 111: 519. 1938.

Using the gravimetric method of Venning the authors studied the excretion of pregnandiol in a case of early pregnancy in which the corpus luteum of pregnancy was removed 58 days after the last menstrual period. Abortion did not take place. Following this operation, the daily urinary content of pregnandiol, an excretion product of progesterone, was determined. Progesterone is probably produced by the placenta, beginning, in this case at least, at about the end of the second month.—Abstract service *J.A.M.A.*

KLEIN, M. Analogous action of testosterone propionate and of progesterone in changes of the endometrium of rabbits (Action analogue du propionate de testostérone et de la progestérone sur les transformations progestatives de l'endomètre chez la lapine). *Compt. rend. Soc. de biol.* 127: 915. 1938.

Three immature female rabbits were primed with 15γ of estradiol benzoate for 8 days, then 2 were castrated. Three adult female rabbits were mated and castrated 18 hr. later. All 6 were then given 2 mg. testosterone propionate daily for 5 days. Histological examination of the uteri of all 6 rabbits revealed progestational proliferation rated + or ++.—E. Cutuly.

KLEIN, M. Simultaneous action of estrone and progesterone in the production and maintenance of vaginal mucification in rodents (Sur l'action simultanée de l'oesterone et de la progestérone dans la production et dans le maintein de la mucification vaginale chez les rongeurs). *Compt. rend. Soc. de biol.* 127: 918. 1938.

Ovariotomy in the pregnant hamster caused cessation of vaginal mucification. Pregnant females which had been castrated and injected daily for 3 to 7 days with 0.5 mg. progesterone did not show mucified vaginae. Vaginal mucification was maintained in pregnant females which had been castrated and treated daily for 3 to 6 days simultaneously with 1γ estradiol and 0.2 mg. progesterone.—E. Cutuly.

KORENCHEVSKY, V., AND M. DENNISON. Cooperative activity of testosterone propionate with Δ^5-androstenediol and oestradiol in male rats. *Biochem. J.* 31: 862. 1937.

Cooperative activity between testosterone propionate and androstenediol was evident in all the sexual organs and in the thymus of castrated rats. Under the conditions specified the addition of estradiol to testosterone propionate slightly increased the weight of the seminal vesicles and considerably increased the weight of the adrenals; the rate of involution of the thymus was slightly, and the gain in body weight considerably, decreased. These results are compared with those previously obtained with other sexual hormones.—*Physiol. Abst.*

KORENCHEVSKY, V., M. DENNISON AND K. HALL. Effects of testosterone and testosterone propionate on adult male rats (compared with those on female rats). *Biochem. J.* 31: 1434. 1937.

In male rats testosterone propionate (T.P.) produced hypertrophy of the secondary sexual organs and hastened the involution of the thymus, whereas testosterone (T.) had little or no effect on these organs. In female rats the effects of T.P. on the development of secondary sexual organs and on the thymus were definite, but much less than those of T. After injection of T., progestational changes were found in the vagina but not in the uterus. Both hormones caused a greater degree of hypertrophy of the female than of the male sexual organs.—*Physiol. Abst.*

LAROCHE, G., H. SIMONNET, E. BOMPARD AND J. A. HUET. The estimation by quantitative tests of the effects of male hormone in the aged (Appréciation par des tests quantitatifs des effets de l'hormone mâle chez les vieillards). *Bull. acad. méd.* 119: 639. 1938.

Seven patients, 53 to 78 years of age, with various symptoms of senility, as arteriosclerosis, acropares-thesia, rheumatism and asthenia were treated for 15 days with 40 or 20 mg. of testosterone propionate daily. All gained weight. The ergographic test showed an average increase of strength of 32%. The heart beat increased from 44 to 55, but the time of sustained work, until fatigue set in, was shortened. The psychomotor time determination showed a decrease of the latency period by 8.6% average. Mental improvement was quite general.—A. E. Meyer.

MARTINS, T., AND J. DO VALLE. Influence of castration on motility of the vas deferens in the rat (Influence de la castration sur la motilité du canal déférent du rat). *Compt. rend. Soc. de biol.* 127: 464. 1938.

The conclusion that in the rat the testis directly or indirectly inhibits contractility of the vas deferens was arrived at from the following results. Vasa deferentia from 14 normal rats, excised, and immersed in Locke or Tyrode solution at 37.5° C. to 39.5° C. and of PH 7.5 to 8 showed little or no spontaneous movement. However, of 30 vasa from rats castrated from 2 to 20 days, 24 subjected to the experimental conditions mentioned, did show spontaneous contractions. Contractions were elicited in vasa from normal or castrated rats by adrenalin, pilocarpine, acetylcholine, eserine, and barium chloride. Vasa from castrated rats injected with estrogens, androgens, or progesterone did not seem to be inhibited.—E. Cutuly.

MOORE, C. R., and DOROTHY PRICE, Some effects of testosterone and testosterone propionate in the rat. *Anat. Rec.* 71: 59. 1938.

Observations on more than 200 male albino rats show that testosterone and t-propionate prevent or repair castration effects on seminal vesicles and prostate gland in young or old male rats, induce precocious secretory responses, maintain the normal growth rate of reproductive accessories, and promote normal prostate-seminal vesicle ratios. Approximately 4 times the daily dose required to maintain histological normality is necessary to promote normal growth of seminal vesicles. The propionate exerted detectable effects approximately 3 days longer than testosterone or androsterone after discontinuance of a series of injections. Testosterone propionate awakens normal copulatory ability within a period of 3 weeks in animals castrated at 30 days of age and treated 6 months later. Seminal vesicles larger than 7-month normal controls are developed in such a period of injection. Testosterone propionate induces retardation in testis development and grave injuries in testes of normal young males. The injurious effects at ages from birth to essential maturity as 80 days are inversely proportional to dosage (0.1 mg. more injurious than 1.0 mg. daily). Testes of mature males receiving daily dosages of 2 mg. are but little affected.—Author's Abst., Wistar Bib. Service.

NOBLE, R. L., Effect of synthetic estrogenic substances on the body-growth of endocrine organs of the rat. *Lancet* 235: 192. 1938.

Synthetic substances representing different types of active compounds in doses of 10–100 mg. per rat (white Wistar) were implanted subcutaneously in the anterior abdominal wall. Control experiments with substances related as closely as possible but, devoid of estrogenic activity, were used in 100-mg. doses in the ovariectomized rat. The animals were weighed at regular intervals. At autopsy, the endocrine organs were dissected, fixed and weighed. The pituitary glands were weighed and dried in acetone. The experiments revealed a marked inhibition of growth in adult rats and that of prepubertal rats. There was an inhibition of the gonadotropic activity associated with the absence of the gonadotropic hormone in the anterior pituitary. Three carcinogenic hydrocarbons tried in a group of 5 male rats did not affect the pituitary secretion or growth.—L. B. S.

NOVAK, E. Masculinizing tumors of the ovary (Arrhenoblastoma, adrenal ovarian tumors). *Am. J. Obst. & Gynec.* 36: 840. 1938.

This is a clinical and pathological study of 11 cases, 6 new, with evidence of the variability of tissues found. The clinical symptoms may be very similar for those tumors with obviously testicular type of tissue and the more rare ones histologically related only to adrenal cortex.—E. L. S.

PERALTA RAMOS, A., AND E. O. COLOMBO. Effects of testosterone propionate (Ensayos biológicos con el propionato de testosterona). *Bol. Acad. Nac. Med. Buenos Aires* 211: 1938.

Injections of 5 to 100 mg. testosterone propionate previous to, or simultaneously with, 100 R.U. of gonadotropic extract (prolan) did not prevent ovulation in the virgin rabbit. Ovarian hormones (estrone and progesterone) neutralized the ovulatory effect of prolan; inhibition of ovulation was complete when the relation estrone : prolan was 18:1. Injection of 5 to 20 mg. testosterone propionate daily beginning 4 days after copulation did not prevent proliferation of the uterine mucosa and implantation of the embryo. The vitelline sac developed normally, but the fetus suffered necrobiosis. This contrasted with the effect of estrone, which prevented development of the vitelline sac and so killed the fetus, because the enlargement of the uterus was insufficient.—J. T. L.

PINCUS, G., AND P. A. ZAHL. The biogenesis of primary sex hormones. The fate of oestrins injected into the rabbit. *J. Gen. Physiol.* 20: 879. 1937.

A method is described for the quantitative isolation of estriol and estrone in rabbit urine in separate fractions free from inactive chromogens. Estriol and estrone are determined colorimetrically. Injected estriol is excreted unchanged, and its excretion is facilitated by the presence of corpora lutea in the injected animal. Estrin-injected animals excrete both estrone and estriol, except when the animals have been hysterectomized or ovariectomized for a long period, when they excrete no estriol. Recovery of injected hormone is never complete, but is always much greater in animals in the luteal phase. The simultaneous injection of progesterone also facilitates estriol excretion, even in those animals normally unable to excrete estriol following estrone injection. It is concluded that the conversion of estrone to estriol takes place in the uterus under the influence of progesterone.—*Physiol. Abst.*

RAYNAUD, A. Formation of a male urethra and penis and absence of vagina in intersexed female mice (Formation d'un urètre mâle, d'un pénis et absence de vagin chez les souris femelles intersexuées). *Compt. rend. Soc. de biol.* 127: 503. 1938.

The author describes the morphology of the urogenital tract in normal newborn male and female mice and in newborn intersexual mice. Without presenting data he discusses the effects produced in fetuses as a result of male hormone injections in pregnant mice during the terminal days of gestation. For example, testosterone propionate administered to pregnant female mice 3 days before parturition caused in the female offspring the formation of a male type urethra and a genital tubercle resembling a penis.—E. Cutuly.

RAYNAUD, A. Sexual behavior of female intersexed mice (Comportement sexuel des souris femelles intersexuées). *Compt. rend. Soc. de biol.* 127: 993. 1938.

Genetically female intersexed mice born from pregnant animals injected with male hormone develop a female genital tract which is normal, except for the absence of the caudal portion of the vagina. The male portion of the genital tract consists of the epididymis which communicates with the Wolffian duct; the latter opens into the urethra which reaches the exterior through a penis. At the age of 2 mo. such intersexes behave sexually as normal females; even though they lack a vaginal orifice, they accept males. If such intersexes are injected with male hormone, they behave sexually as normal males and will copulate. Intersexes cannot reproduce, because as females they lack a vagina, and as males they lack testes.—E. Cutuly.

RÉGNIER, V. Effect of folliculin on the comb of normal cocks (Action de la folliculine sur la crête des coqs normaux). *Compt. rend. Soc. de biol.* 127: 519. 1938.

Estradiol benzoate at a dose level of 2 mg. or less a week (3 injections) was injected into birds of 3 age groups (1 mo., 4 to 5 mo., and adults). This treatment at first caused retarded comb growth in the infantile birds and regression in the older birds. Later, however, the combs began to grow anew despite continued treatment. It was suggested that estradiol temporarily decreased male hormone production by the testes.—E. Cutuly.

REYNOLDS, S. R. M., The activation of uterine muscle by estrin and its relation to uterine growth. *Am. J. Obst. & Gynec.* 36: 437. 1938.

This paper presents a series of concepts based on previously published work of many authors. The order of effects of estrin on a quiescent uterus is thought to be production of local vasodilator substance, with increased blood flow, followed during the next several hours by an increasing muscle metabolism, after which rhythmic contractions begin to appear and become increasingly vigorous, thereby facilitating the local circulation by increasing the blood flow and the formation and flow of lymph, ultimately leading to growth of the organ.—E. L. S.

ROCK, J., J. REBOUL AND J. M. SNODGRASS. Electrical changes associated with human ovulation. *Am. J. Obst. & Gynec.* 36: 733. 1938.

By means of indifferent electrodes on different parts of the lower abdomen, or one outside and a

second within the vagina, potential differences were recorded at intervals of 15 min. for periods of hours to days during the part of the cycle when ovulation was expected. In several cases relatively sudden changes of potential, returning to previous levels, suggested that ovulation had occurred. Laparotomies followed within a few hours, indicating that ovulation had taken place. In a few cases there was maturation of ova without ovulation. Some of the evidence suggests that the electrical changes precede the actual liberation of the ovum. So far as it goes the evidence is in agreement with the current views as to the time of ovulation with reference to the next ensuing menstrual flow.—E. L. S.

RUBINSTEIN, H. S. Induction of sexual maturity in the genitally hypoplastic adult through the use of testosterone propionate. *J.A.M.A.* 111: 1818. 1938.

Injections of testosterone propionate were given to a genitally hypoplastic man 6 mo. after cessation of response to treatment with gonadotropic substance from the urine of pregnancy. This substance was injected intramuscularly in 5 mg. doses 3 times a week from the latter part of July to Nov. 1937. Dosage was then increased to 25 mg. 3 times a week. In Jan. 1938 the genital condition improved to such an extent that the patient married, cohabited and ejaculated live spermatozoa. While the voice became somewhat deeper, distribution of hair and the initial beardlessness remained unchanged. Laboratory studies on the effect of this hormone on the testis showed a stimulation and proliferation of germinal epithelium but failed to reveal a hastening in maturation of the testes. It was concluded, therefore, that in this case secondary effects may have been responsible for first causing the germinal epithelium to reach such a stage of maturation and that further treatment with testosterone resulted in the proliferation and discharge of mature and live spermatozoa.—Abstract service *A.M.A.*

RUBINSTEIN, H. S., and H. M. RADMAN, A comparative study of the gross and microscopic effects of follicle simulating hormone and anterior pituitary sex hormone on the rat testis. *Am. J. Physiol.* 122: 319. 1938.

Mature and immature male rats were injected with either anterior pituitary sex hormone or urinary FSH. Both hormones produced testicular descent in the immature rats. Urinary FSH produced testicular enlargement, interstitial tissue increase and proliferation of germinal epithelium in both mature and immature animals. Anterior pituitary sex hormone produced a lesser degree of interstitial tissue increase and germinal epithelium proliferation in both mature and immature rats, but testicular enlargement only in the mature animals.—C. Duncan.

RUBOVITS, W. H., AND W. SAPHIR. Intersexuality. *J.A.M.A.* 110: 1823. 1938.

The authors studied clinically, physiologically and psychologically a case of intersex, which, according to the older nomenclature, may be classified as pseudohermaphroditism masculinus. Although a laparotomy was not performed and thus the absence of ovaries was not definitely established, this diagnosis would hold true even if some rudimentary ovaries were present, for the presence of both ovaries and testicles in one individual cannot be called true hermaphroditism unless there is definite evidence of gonadal function, such as the presence of both ova and spermatozoa. Microscopic evidence of testicular tubular atrophy and absence of spermatogenesis was enough to rule out the diagnosis of true hermaphroditism and to confirm again the fact that true hermaphroditism never has been found in man. The patient who had been brought up to be a woman, experienced intense libido toward the male sex. The question arises whether sexual desire is a manifestation rather of environmental influences than of gonadal function. The fact that sexual tension was relieved by orchidectomy could be evaluated in favor of the assumption that the gonads are responsible for sexual desire even if expressed toward the same sex. The common embryonic origin of ovaries and testes, the near chemical relationship of androgenic and estrogenic substances, which are found excreted by both males and females, and the favorable effects of castration in homosexuality are factors that would interpret libido as a specific manifestation of gonadal activity, no matter toward which sex they are expressed and no matter as to the environmental influences.—R. G. H.

SAVAGE, J. E., H. B. WYLIE and L. H. DOUGLASS, A chemical test for pregnancy applied to the determination of estrin in the urine of normal and toxemic patients in the last trimester of pregnancy. *Am. J. Obst. & Gynec.* 36: 39. 1938.

Extending an earlier paper this shows decreased estrin excretion in late toxemias of pregnancy. A 4-hour colorimetric test for urinary estrin is described and employed. The reduction in estrin is nearly the same in chronic nephritics and pre-eclamptics. Use of 10,000 I.U. estrin daily for 3 days in 9 cases gave no certain results.—E. L. S.

SCHERF, D., The electrocardiogram in disturbances of ovarian function (Über das Electrokardiogram bei Storungen der Ovarialtatigkeit). *Klin. Wchnschr.* 17: 44. 1938.

In women complaining of menopausal circulatory disturbances, there was frequently noted an

angina-like syndrome, which appeared not on exertion but while the patient was at rest, and which lasted only for a few moments. In such women the EKG showed a depression of the S-T segment below the line, especially in lead II, and a decreased T wave. With estrin therapy, the EKG returned to normal, together with improvement in symptoms. In one patient, the administration of nitroglycerin corrected the EKG three minutes after the medication, but of course the effect was of short duration. In view of the vasodilating effect of estrin, the author advances several reasons for considering the EKG abnormality as due to some myocardial damage on the basis of functional coronary inadequacy.—M. H. F.

Sempronj, A., E. Morelli and A. Dansi, Some products of polymerization of p-oxypropenylbenzene as compared by their estrogenic activity (Su alcuni prodotti di polimerizzazione del p-ossipropenilbenzolo in rapporto alla loro attivita estrogena). *Biochim. e. terap. sper.* 25: 153. 1938.

Spontaneously polymerized anol (oxypropenylbenzene) is active in doses between 14 and 20 mg. A dimeride of anol was isolated as a benzoyl compound and found to be inactive at relatively high dosage. The action of formic acid led to higher polymerides which were also inactive. The theory is advanced that the active substances are dimerides, although of a special type of chemical constitution similar to the highly active dihydroxy α, β diethylstilbene. The formation of this substance has not been proved.—A. E. M.

Shute, E., Determination of the estrogenic substance in blood serum by means of an estimation of the antiproteolytic power of the serum. *Am. J. Obst. & Gynec.* 35: 970. 1938.

Serum plus a phosphate-buffer are incubated at 55° C., cooled, and incubated with trypsin at 42° C. Aliquots are titrated with alkali at 10-minute intervals for 40 to 60 minutes. The details are to some extent empirically determined. There is acid production, followed later by 'reversal.' Delay in the increase of acidity is interpreted as evidence of excess of estrin in the serum. Addition of estrin to serum samples produced this result. There is discussion about the relation of estrin excess to deficit of vitamin E.—E. L. S.

Smith, G. Van S., and O. W. Smith. Observations concerning the metabolism of estrogens in women. *Am. J. Obst. & Gynec.* 36: 769. 1938.

Chemical means have been used to identify and quantitatively estimate in urine samples the pregnandiol, estriol, estrone, and a third estrogenic material which has properties consistent with its being estradiol. The results indicate that the conversion of estradiol into estrone is reversible, but that conversion of estrone to estriol is not reversible. The presence of the uterus and of progestin are necessary conditions for the formation of estriol. Progestin appears to protect estrogens against destruction, allowing greater utilization, and leaving more for excretion. The deficiency of progestin is followed by reduction of estriol formation and excretion, and increased destruction of estrogens, with decreased excretion of estrins, but relatively more estradiol excretion in proportion to estrone loss. Endometrial bleeding is associated with increased production and increased destruction of estrogens, accompanied by progestin deficiency. Dysfunctional flowing (without progestin action in the cycle) is an exaggerated form. The authors postulate, as a substitute for the usual estrin privation theory of menstrual flow, that the vascular phenomena of flow are induced by a toxic concentration in the uterus of non-estrogenic products from the destruction of estrogens. (While progestin is available the less toxic estriol is formed.) The findings in pre-eclampsia resemble those in a progestin-deficient metabolism of estrogens. Since earlier work had led to the conclusion that there was estrogen deficiency also in these toxemias of the third trimester, preliminary trials have been made with estradiol and progestin therapy, with some promise of value.—E. L. S.

Tapella, P. A., Effect of follicular hormone on compensatory hypertrophy of the ovary (Acción de la hormona follicular sobre la hipertensión compensadora del ovario). *Rev. Soc. argent. de biol.* 14: 31. 1938.

Adult female rats received 2.5, 10, 20, 50, 200, 500 I.U. daily of estradiol in oil for 6 weeks, commencing 2 days after removal of one ovary and half the other ovary. At the end of the experimental period they were killed and the remaining ovarian fraction weighed. Control untreated rats, and those receiving 2.5 I.U. daily showed compensatory hypertrophy of the ovarian fragment. In those receiving 10 to 500 I.U. daily the ovarian fraction was considerably reduced in weight, comparing it with the weight of the half removed on operation. Microscopical sections showed numerous atresic follicles and no corpora lutea. The hypophysis of those receiving 500 I.U., was considerably enlarged; the genital tract was also enlarged due to distension with a clear secretion, without signs of infections. It is deduced that a sufficiently intense and prolonged treatment with the follicular hormone inhibits compensatory hypertrophy of the ovary.—J. T. L.

THOMPSON, W. O., AND N. J. HECKEL. Precocious sexual development from an anterior pituitary-like principle. *J.A.M.A.* 110: 1813. 1938.

In 23 patients treated for undescended testis with the APL principle it was observed that genital growth was produced in 18 and that in 14 it was marked. In 2, aged 7 and 9 years, a condition resembling premature puberty resulted. The penis became as large as that of an adult. There was an increase in the size of the scrotum and prostate, a growth of pubic hair, a marked increase in frequency of erections, a change in the pitch of the voice, and in one a growth of hair on the sides of the face. Similar changes were produced in a 4-year-old boy out of a total of 12 patients with hypogenitalism, and less marked growth in 8 others. The boy showing changes simulating premature puberty was of normal body contour but had an atrophic left testis. All boys showing genital growth became more masculine. It should be pointed out that in some of the patients with undescended testes and hypogenitalism, who failed to show any growth the dose may have been inadequate, there being several examples in the series of failure of growth with a small dose and a well marked growth with a larger dose. In contrast to the marked growth of other parts of the genitalia, the testes showed very little change in size. Descent was produced in only 23% of the total number of undescended testes, showing that genital growth may occur without descent of the testis. The treatment for undescended testes with the anterior pituitary-like principle should be stopped before genital growth becomes marked. If this rule is followed, it would appear that in the present state of knowledge its routine use is justifiable and desirable.—Abstract service, *J.A.M.A.*

TURNER, C. L., The reproductive cycle of *brachyraphis episcopi*, an ovoviviparous poeciliid fish, in the natural tropical habitat. *Biol. Bull.* 75: 56. 1938.

In the Panama canal zone (9° N. latitude), a total of 1139 specimens were collected during 11 months of the year. Of these, 442 females and 288 males were examined. No seasonal variation in the production of maturity in the males was noted. In the females, reproduction occurs in short cycles which are continuous and not seasonal.—E. L.

VAN DYKE, H. B., AND R. C. LI. The secretion of progesterone by the cat's ovary following the formation of corpora lutea due to the injection of anterior pituitary extract or prolan. *Chinese J. Physiol.* 13: 213. 1938.

The secretion of progesterone by corpora lutea formed after prolan or A.P. injection was tested by the epinephrine response of the uterus in 36 cats. Relatively enormous doses of hormone are necessary to produce ovulation with subsequent luteinization in the cat. Sample segments of uterus were removed aseptically and with minimal damage to the uterine blood supply; they were suspended in van Dyke-Hasting's bath-fluid and tested with various concentrations of epinephrine. The response was negative for the first few days after ovulation; from 5 to 12 days there was a contraction; then again negative until the 20th day when the response was relaxation. In 3 cases, estrone injection lengthened the contraction (progestrone) phase. No consistent response to the oxytocic post-pituitary principle was found in the cat. Photographs of ovaries, ovarian sections and kymographic records are included.—R. L. Z.

VESELL, M., Cyclic treatment of a case of secondary amenorrhea of ten years' duration. *Am. J. Obst. & Gynec.* 35: 1067. 1938.

A 31-year-old sterile woman, with 10 years of amenorrhea, was given APL 100 U daily for 5 days, then for 5 months 500 U at similar intervals each month. Menses returned, therapy was continued, and after 10 months she conceived. She went to term without further therapy.—E. L. S.

VEST, S. A., JR., AND J. E. HOWARD. Clinical experiments with use of male sex hormones. Testosterone propionate in hypogonadism. *J. Urol.* 40: 154. 1938.

The authors employed testosterone propionate in five cases of primary testicular insufficiency due to castration, atrophy following trauma or an inherent defect in the testes and in one case of gonadal insufficiency secondary to demonstrable disease of the pituitary. Because of the known wide variation in the age of sexual maturation in man, their patients were only those with hypogonadism who were well past the normal age period of adolescence. Androgen seems to be a satisfactory replacement therapy for hypogonadism in the human being. It produces profound anatomic changes resulting in the proportionate growth of the phallus, scrotum, seminal vesicles and prostate, as well as development of pubic, axillary and extremity hair. There have been laryngeal changes, the appearance of considerable prostatic secretion and an ejaculum with coitus, and marked changes in the skin. There have been, in addition, changes in the general appearance, with improvement in the personality content. It has induced libido and potentia in persons in whom these had not existed previously, and restored normal sex life in a patient who was impotent following castration. No evidence of increase in tolerance to the drug has been observed.—Abstract from *J.A.M.A.*

VILLARET, M., L. JUSTIN-BESANCON AND A. RUBENS-DUVAL. Effect of testosterone propionate in a case of post-puberal eunuchoidism (Remarques sur les effets du propionate de testostérone dans un cas d'eunuchisme post-pubère). *Compt. rend. Soc. de biol.* 127: 599. 1938.

A eunuchoid, 21 years of age, 1.62 m. tall and weighing 55 kg. was treated daily with 10 to 30 mg. of testosterone propionate for a period of 6 mo. Early results included erection of the penis and turgescence and flushing of the scrotum. After 2 mo. the voice dropped and hair began to develop on the pubis and in the axillae. After 6 mo. of treatment height and weight had increased to 1.66 m. and 63 kg., respectively, but radiological studies indicated that epiphyseal fusion had not been induced.—E. Cutuly.

WILSON, L., and R. KURZROK, Cystic endometrial changes in ovulatory cycles. *Am. J. Obst. & Gynec.* 36: 302. 1938.

From a study of over 1000 endometrial biopsies there are reported 5 in which there is evidence of predominantly an estrin effect plus some signs in endometrium of glands, or often chiefly in the stroma, of the action of progrestin. The authors differentiate this from the iregular development of the progestational effect in the early part of the normal third week. They believe it due to prolonged estrin effect followed by ovulation and the activity of progestin. Attempts to produce the result by use of massive doses of progestin were unsuccessful, even in cases with cystic glandular hyperplasia, but in normal cycles they were able to reproduce the picture by delaying the menstrual cycle with massive doses of estrin during the first two weeks of the cycle.—E. L. S.

WOLLNER, A., The histologic correlationship of endometrial and cervical biopsies. *Am. J. Obst. & Gynec.* 36: 10. 1938.

Cervical and endometrial biopsies were obtained at the same sitting from 44 women, covering the various stages in the menstrual cycle. There is apparently a synchronous cycle in both types of tissue, which is illustrated. A number of advantages are claimed for the procedure of cervical biopsy, especially for office procedure and repeated examinations of the same woman.—E. L. S.

WOODHAM, C. W. B., Hyperplasia of the male breast accompanying malignant disease of the testicle treated by x-rays. *Lancet* 235: 307. 1938.

Three cases (2 others are mentioned) of enlargement of the breast in males aged 32, 37 and 30 years respectively, following the removal of a teratoma of one testicle, and irradiation of the other testicle, are described. One unilateral and 2 bilateral enlargements of the breast caused local discomfort and pain. In none was any secretion obtained from the nipple, and palpation revealed a granular feeling characteristic of a chronic mastitis. Histologically, there was hyperplasia with great dilatation of the duct system and an increase in the stroma.—L. B. S.

ZONDEK, B. Cutaneous application of follicular hormone. *Lancet.* 1: 1107. 1938.

Zondek used tincture of estrogen percutaneously followed by intramuscular injections of progesterone in the treatment of 18 instances of primary amenorrhea in 12 patients. After the percutaneous treatment with estrogen (from 10,000 to 30,000 M.U.) intramuscular injections of from 8 to 10 RB.U. of progesterone were given daily for 5 days. Tincture of estrogen appears more effective than that of estradiol benzoate. Rubbing in 5 cc. of an estrogenic solution twice a day appears to be the best method. Bleeding was induced 10 times. In 3 of the 8 failures menstruation was later induced by injections of estrogen so that the percutaneous route seems still to be somewhat inferior to the subcutaneous; but its simiplicity makes it agreeable to the patient, especially when treatment has to be continued permanently. In 10 cases of secondary amenorrhea (13 cycles), varying in duration from one to 14 yr., bleeding was induced 7 times. Local treatment with estrogenic tincture is worthy of consideration in arthritis ovaripriva.—Abstract from *J.A.M.A.*

ZONDEK, B., and E. BERGMANN, Phenol methyl ethers as oestrogenic agents. *Biochem. J.* 32: 641. 1938.

The methyl ethers of estrone and estradiol are almost as active as the parent compounds. Certain essential oils, such as fennel oil, anise oil, eucalyptus oil, are estrogenic; this is due to their content of such phenolic ethers as anethole, anisole, veratric aldehyde, anol. Some of these substances are far more active in immature female rats than in adult castrated mice.—D. L. T.

ZUCKERMAN, S. Oestrone threshold of uterus of Rhesus monkey. *Proc. Roy. Soc., London, s. B.* 123: 441. 1937.

Spayed Rhesus monkeys were used. Different animals vary in respect to the least amount of estrone, administered over 14 days, required to influence the endometrium, so that cessation of injections is followed by uterine bleeding. The interval between cessation of injections and onset of bleeding and the duration of bleeding vary from time to time and from animal to animal. The bleeding which normally occurs after cessation of a course of injections can be averted by recommending injections of estrone before or immediately after the middle of the interval between cessation of injections and onset of bleeding;

this is taken to mean either that the effects of a series of injections are prolonged some days after their cessation or that the processes leading to endometrial breakdown are reversible in their earlier stages. Daily injections of an amount of estrone well above threshold value were given for a year; during this period no uterine bleeding occurred. Daily injections of amount of estrone just about threshold value were given for a year; uterine bleeding occurred 8 times at intervals of from 5 to 7 weeks; this is taken to mean that in spayed (and presumably in normal) animals there is a rhythmical rise and fall in estrin threshold, for reasons unassociated with cyclical changes in the gonads.—*Physiol. Abst.*

ZUCKERMAN, S. Part played by oestrogenic hormone in menstrual cycle. *Proc. Roy. Soc. London, s. B.* 123: 457. 1937.

Increasing doses of estrone were given to spayed Rhesus monkeys for 14 days; on the 15th day the daily dose was reduced to a constant level below threshold level, at which it was maintained. If uterine bleeding began during the low level of injections, the experiment ended and a new one was begun on the 2nd day of bleeding. The interval between and including the 1st day of injection and the 1st day of bleeding is referred to as an 'artificial cycle'. If bleeding did not occur within 40 days of the commencement of the experiment injections were stopped and the animals not injected again until after the occurrence of estrin-withdrawal bleeding; a new experiment was then begun. The 'control cycle' is the interval between and inclusive of the 1st day of injections of estrone and the 1st day of bleeding, when no estrone is given after the 14th day. The average length of 39 'artificial cycles' was 26.7 days, that of 39 'control cycles' was 22.3 days; thus reduction of the daily dose of estrin to below threshold level after the 15th day, instead of omitting to give it at all, definitely delays uterine bleeding. The extent of the fall of estrogenic stimulation on the 15th day seems to have no effect on the readiness with which bleeding occurs, but the amount of estrone given during the first 14 days has a slight effect on bleeding; this occurs sooner when the total amount given in the first 14 days is relatively small, and when the level to which the dose is dropped on the 15th day is in the lower part of the threshold range. Eighteen of 20 animals previously tested and found to have high estrone thresholds did not bleed on doses higher than their thresholds. Sixteen of 18 animals previously tested and found to have low thresholds bled on doses which were from 2 to 5 times their tested thresholds. It is concluded that the threshold fluctuates over a wide range, and that some animals had been tested at the bottom and some at the top of their curve of estrone sensibility; this is related to the previously reported finding that there are cyclical fluctuations in the estrone threshold.—*Physiol. Abst.*

HYPOPHYSIS

MARKS, H. P., AND F. G. MARKS. The influence of anterior pituitary extracts, injected either with or without insulin, on the glycogen contained in the livers of fasting young rabbits. *J. Physiol.* 93: 61. 1938.

The rise in the liver glycogen which usually follows the administration of insulin to fasting young rabbits was prevented by the injection of prolactin or thyrotropic fractions into 16 rabbits, but not by crude saline pituitary extracts (8 rabbits). This active factor may be identical with the glycostatic factor of Russell; but different from Young's glycotropic factor, prolactin, gonadotropic and thyrotropic factor. There was also a great increase in the weight of the liver following administration of all 3 extracts; due to the presence of extra total water and fat as well as glycogen.—J. W. Jailer.

MELVILLE, K. I. Electrocardiographic and blood pressure changes induced by posterior pituitary extract (postlobin-V) and the influence of ephedrine thereupon. *J. Pharmacol. & Exper. Therap.* 64: 86. 1938.

Electrocardiograms and blood pressure were taken of anesthetized and unanesthetized dogs. Injection of postlobin-V, in dosages of 1 to 2 U per kg. produced diphasic rises in blood pressure. During the fall in B.P. there was slowing of the heart rate and accentuation of both the R and T deflections with no evidence of prolongation of P-R interval or block in conduction. In addition to these effects, the P-wave was reversed after vagotomy. Ten minutes after injection, the heart rate and contour of electrocardiogram were normal. In the unanesthetized dogs, injection of postlobin-V caused an increased height of the T-wave and some temporary slowing. In vagotomized animals, rapid bigeminal rhythm, attributed to the effect of high B.P., was produced. In both sets of animals, the previous administration of ephedrine (2 mg. per kg.) prevented the fall in blood pressure and characteristic alterations of the T-wave. Changes in T-wave are ventricular effects resulting from the anoxemia produced by coronary constriction which is also responsible for the slowing and P-wave changes. The author believes that postlobin-V has no primary effect on either the myocardium or conducting mechanism of the heart but all such effects are due to its coronary-constricting action.—E. L.

METZ, M. H., AND R. W. LACKEY. Peptic ulcer treated by posterior pituitary extract. *Texas State J. Med.* 34: 214. 1938.

The use of posterior pituitary preparations in treatment of peptic ulcer was suggested by the

observation of polyuria associated with the ulcer syndrome. Forty-two individuals with peptic ulcers have been treated with posterior pituitary preparations with satisfactory clinical results in 40. The duration of treatment averaged 28 days, but subjective improvement was present after one to eight days. Posterior pituitary powder intransally has proved the most satisfactory mode of administration. The hypothesis that victims of peptic ulcer frequently exhibit posterior pituitary deficiencies is discussed.—Authors' Summary.

Munoz, J. M. Effect of anterior pituitary extract on blood fats (Action de l'extrait antérohypophysaire sur les graisses du sang). *Compt. rend. Soc. de biol.* 127: 156. 1938.

The A.P. extract increased fatty acid, cholesterol, and phospholipid levels in the blood.—E. Cutuly.

Pasqualini, R. Q. The water metabolism of *Bufo arenarum* Hensel. Effect of pituitary extracts (Estudios sobre el metabolismo hidrico en el *Bufo arenarum* Hensel). *Rev. Soc. Argent. de Biol.* 14: 260. 1938.

Posterior pituitary lobe extracts had antidiuretic activity on normal toads. Small doses did not modify cutaneous absorption of water, large doses increased it (this is considered a toxic not a physiologic effect). Polyurea in the hypophysectomized toads was controlled by injections of bovine pituitary (posterior lobe) extract, or the emulsion of posterior lobe of toad pituitary. While under the effect of the extract no change in renal glomerular activity was observed; with larger doses, specially when given intravenously the stoppage in the circulation was always of shorter duration than the antidiuretic effect. In animals under the oliguric effect of the extract, insulin injected intraperitoneally was eliminated as by the controls. It is deduced that the oliguric effect of posterior pituitary extract in hypophysectomized toads is due to an increase in reabsorption by the tubuli.—J. T. L.

Plummer, D. E., and J. R. Jaeger. Pituitary cachexia (Simmonds' disease). Report of a case with autopsy. *Arch. Neurol. & Psychiat.* 40: 1013. 1938.

The case is somewhat unique in that the etiology is rather clearly a glioblastoma multiforme which was observed postmortem in structures adjacent to the pituitary gland and exerting pressure on it. The patient made rapid improvement during a month of treatment with gonadotropic substance from P.U. The author comments on the probability that this disorder in mild form may at times be mistaken for other conditions.—R. G. H.

Rasmussen, A. T., and A. A. Nelson. Pars intermedia, basophil adenoma of the hypophysis. *Am. J. Path.* 14: 297. 1938.

Two cases of basophil adenoma originating from the pars intermedia are described. In 1 case the only symptom at all referable to the hypophysis was high blood pressure. On account of the age of this individual (77 yr.) the association of the adenoma with the hypertension is questionable. In the second case a number of the major characteristics of pituitary basophilism (adiposity, striae atrophicae, hirsuties, high blood pressure, florid face) were present. In neither case were there hyaline changes in the basophils. There was considerable diffuse invasion of the neural lobe by basophils in both cases. The great bulk of data, however, fail to indicate that there is any direct significant correlation between this invasion and hypertension.—Authors' Summary.

Rea, C., and Hoover, P. A. Simmonds' disease. With case report. *Pennsylvania M. J.* 42: 27. 1938.

The case reported occurred in a white married woman of 29, with symptoms of Simmonds' disease extending over a period of 16 yr. The first 'crisis,' occurred in January, 1932. From this she made a rapid recovery upon the administration of 5 U of insulin 3 times daily. A weight gain of almost 100 lbs. took place in less than a year. Within 6 mo. the symptoms returned and a second crisis developed in December, 1936. Insulin therapy this time had no effect. Upon beginning substitution therapy with A.P. liquid an immediate and remarkable recovery began. All symptoms and clinical signs disappeared, with a weight gain of approximately 80 lb. This case would seem to suggest that the pancreas was definitely involved in the syndrome when the patient was under attention in 1932, and that subsequently the anterior pituitary was predominantly at fault. The authors remark that apparently this woman is now well, but raise the question of the permanency of recovery.—I. B.

Robertis, E. de. Cytology of the liver in pancreatic and hypophyseal diabetes (Cytologie du foie dans les diabètes pancréatiques et hypophysaire). *Compt. rend. Soc. de biol.* 127: 158. 1938.

The liver of dogs injected with the A.P. extract contained enlarged cells with slightly increased glycogen content but greatly increased fat content. Details of cytological changes are given.—E. Cutuly.

Silvette, H., and S. W. Britton. Renal function in the opossum and the mechanism of cortico-adrenal and post-pituitary action. *Am. J. Physiol.* 123: 630. 1938.

Cortico-adrenal extract was effective in producing changes in renal excretion in both normal and adrenalectomized opossums; it was slightly more effective intraperitoneally than by mouth. However adrenalin injections were without effect. Post-pituitary solution produced a typical antidiuretic effect

when injected into water fed opossums, but was ineffective when the urine was highly concentrated. Cortico-adrenal extract to normal and adrenalectomized opossums increased the fluid excretion, but decreased the chloride and urea eliminated per cc. of urine. The interrelation of the pituitary and adrenal in fluid regulation is discussed.—Lena Lewis.

STUTINSKY, F. Regenerative zones in the hypophysis of adult frogs (Sur les zones de régénération dans l'hypophyse de la grenouille adulte). *Compt. rend. Soc. de biol.* 127: 184. 1938.

Two types of regeneration are described. One is by so-called endocytogenesis. The other is said to be by differentiation of cells from cellular cords. These cords are reported to consist of a germinal center of indifferent chromophobic cells which apparently divide amitotically and, as they progress centrifugally, differentiate successively from the indifferent state to eosinophiles and finally to basophiles.—E. Cutuly.

UNGER, G. The course of nerve fibers to the hypophysis (Sur le passage de fibres nerveuses a destination hypophysaire). *Ann. d'anat. path.* 15: 815. 1938.

By electric stimulation of the sympathetic trunk, sympathetic ganglia and various spinal nerve roots of cats and dogs, it appeared that the hypophysis is excited (as judged by an increase in the oxytocic power of the cerebrospinal fluid) by fibers from the dorsal root of the second cervical spinal nerve passing through the superior cervical sympathetic ganglion and upward in the internal carotid plexus.—A. T. R.

WEGELIN, C. The hypophysis in Basedow's disease (L'hypophyse dans la maladie de Basedow). *Ann. d'anat. path.* 15: 703. 1938.

The size and histological features of the hypophyses in 20 cases of Basedow's disease were within normal limits. There were no evidences of hyperfunction of the adenohypophysis nor any alteration in the neurohypophysis as to basophilic infiltration, colloid and pigmentation. Young guinea pigs injected intra-abdominally with acetone extracts of 18 human hypophyses (4 from cases of exophthalmic goiter) indicated no difference between the extract of Basedowians and of the rest. These negative findings correlate with clinical observations that the thyrotropic hormone is diminished in the blood of patients with Basedow's disease. A small basophilic adenoma was present in one case without symptoms of pituitary basophilism.—A. T. R.

WHITE, H. L., AND P. HEINBECKER. Observations on creatinine and urea clearances on response to water ingestion and on concentrating power of kidneys in normal, diabetes insipidus and hypophysectomized dogs. *Am. J. Physiol.* 123: 566. 1938.

Hypophysectomized and diabetes insipidus dogs show no significant alterations from normal in the time relations of their blood dilution and urine output responses to 3% body weight of water. During transitory phase of polyuria the creatinine clearance is unchanged, the urea clearance slightly elevated, both are normal during the normal interphase. Two to 4 weeks after beginning of permanent polyuria the creatinine clearance is 50% of normal. It gradually returns to a nearly normal level 6 mo. after permanent polyuria. The urea clearance fall is less than the creatinine during permanent polyuria. The urea creatinine clearance ratio may be as high as 0.8. On administration of anterior lobe extract the clearances rise, the urea more markedly, so that urea creatinine ratio is 0.8 to 0.9. The ability of hypophysectomized dogs to tolerate water deprivation is normal but that of diabetes insipidus dogs greatly decreased.—Lena Lewis.

PANCREAS

JOACHIM, H., AND M. M. BANOWITCH. A case of carcinoma of the islands of Langerhans with hypoglycemia. *Ann. Int. Med.* 11: 1754. 1938.

A case of spontaneous hypoglycemia is reported in which the entire syndrome ran its course in 2 mo. At operation a tumor of the pancreas which proved to be a carcinoma of the islets of Langerhans was found.—F. A. H.

KEYS, A. The effects in man and dogs of massive doses of insulin on the composition of the blood serum. *Am. J. Physiol.* 123: 608. 1938.

Physically normal adult males with schizophrenia were injected with 20 to 130 U of insulin. In all cases (10 studies) the following changes in the composition of the blood serum were observed at the height of the insulin reaction. *a*), A moderate increase in the concentration of the proteins, range 1.5 to 10.4% mean 4.2%. *b*), A marked decrease in the concentration of K, range 12.7 to 26.8%, mean 18.4%. In all but one case the concentration of N.P.N. in the serum decreased significantly; the average decrease was 12.4%. At least a part of this change involved substances other than urea. In all but one case the concentration of Na in the serum rose slightly; the average increase was 1.9%. Studies on dogs yielded results very similar to those obtained on man. The changes in K concentration in man are ascribed in large part to hyperactivity of the adrenal glands provoked by the hypoglycemia.—Author's Abstract.

KLATSKIN, G. The response of diabetics to a standard test dose of insulin. *J. Clin. Invest.* 17: 745. 1938.

To test the diametrically opposite conclusions of Himsworth and MacBryde about the advantage of high carbohydrate diet for insulin-sensitive or insulin-resistant patients, 50 diabetics were chosen from a larger group because of cooperation. They had been under control for 5 or more months before the tests were made. The MacBryde intravenous test dose was used. The division of patients into resistant and sensitive types appears to be arbitrary, for the obvious best correlation is that the fall in blood sugar varies directly with the elevation of the blood sugar at the beginning of the test. There seems to be no correlation between the clinical characteristics of the individual cases or their response to the types of diet and the sensitivity by this test.—E. L. S.

MARBLE, A. Insulin resistance. Report of a case of marked insensitiveness of long duration without demonstrable cause. *Arch. Int. Med.* 62: 432. 1938.

A patient with chronic rheumatoid arthritis and with marked insensitiveness to insulin has been observed closely since July 1935 when she was 35 years old. The insulin requirement has varied from 240 to 675 U daily. Complications included marked general glandular enlargement, slight to moderate hepatomegaly and splenomegaly, and marked eosinophilia. The latter is attributed to the large doses of insulin. Lipodystrophy at the site of the injections of insulin has been a problem. Injections of insulin into areas of tumefaction and scarring, produced by multiple previous injections, were less effective than injection into a normal area. Control of the diabetic condition was followed by a gain in weight and strength. Attempts to demonstrate a pituitary, adrenal, thyroid or gonadal influence as being responsible for the resistance to insulin were unsuccessful.—I. B.

MILLER, S. P., AND A. C. KUYPER. The relation of insulin and adrenalin to uric acid excretion in the rabbit. *Am. J. Physiol.* 123: 625. 1938.

The injection of insulin or adrenalin into rabbits increases the amount of uric acid excreted. Insulin exerts its effect indirectly by lowering the blood sugar and causing mobilization of adrenalin. Adrenalin appears to affect purine metabolism first but protein catabolism as a whole may also be stimulated. In the human organism insulin and adrenalin administered in therapeutic amounts, have no significant effect on the rate of excretion of uric acid.—Authors' Abstract.

NEUHOFF, F., AND S. RABINOVITCH. Protamine zinc insulin. Clinical observations and comparative analysis of blood sugar curves obtained with use of protamine zinc insulin and with regular insulin. *Arch. Int. Med.* 62: 447. 1938.

Fluctuations in the blood sugar values for 12 diabetic patients who were first treated with regular insulin and then with protamine zinc insulin are presented. In 50% of these cases, regulation was successful with 1 daily injection of protamine insulin, and the total requirement for insulin was reduced from 42 to 17%. In the remainder, satisfactory regulation could not be obtained with either 1 or 2 doses of protamine insulin. The protamine insulin had a more gradual and prolonged lowering effect on the blood sugar content than did regular insulin hydrochloride. The lowest blood sugar level during the 24-hr. period occurred *a*), after 20 hr. when the protamine insulin was injected at 7 A.M. and *b*), after 5 hr. and in 1 case after 12 hr. when it was injected at 10 P.M.—I. B.

RICKETTS, H. Carbohydrate tolerance after protamine insulin: its bearing on the physiology of insulin secretion. *J. Clin. Invest.* 17: 795. 1938.

In severe diabetics when protamine insulin is adequate to maintain fasting blood sugar in the normal range, it is unable to prevent post-prandial hyperglycemia. In milder diabetics the post-prandial glycemic reaction under these conditions approaches the normal response. Depancreatectomized dogs resemble severe human diabetics in this respect. The results do not support Soskin et al. in the hypothesis that the liver reduces hyperglycemia without the aid of extra insulin after the ingestion of sugar. The author repeated Soskin's experiment in a diabetic under use of protamine insulin, adding the continuous intravenous injection of insulin and glucose to maintain a steady state of normoglycemia. Oral glucose tolerance test then gave a normal response, whereas the same patient with protamine insulin but without the intravenous therapy gave a diabetic type of response. This suggests that the conditions of the experiment are too unusual to determine the point at issue. The results are held therefore to be in favor of the theory that the ingestion of carbohydrate stimulates insulin secretion by the pancreas.—E. L. S.

SCOTT, D. A., AND A. M. FISHER. The insulin and zinc content of normal and diabetic pancreas. *J. Clin. Invest.* 17: 725. 1938.

Comparing 14 normal and 18 diabetic pancreases the insulin is found one fourth as much and the zinc one half as much in the diabetic pancreas. There is no marked difference of zinc in liver of the diabetics as compared with the normals. Normal human pancreas insulin content is similar to that in cats and cows, 1.7 I.U. per gram.—E. L. S.

WIENER, H. J. Diabetic coma requiring an unprecedented amount of insulin. Report of a case manifesting extreme insulin resistance. *Am. J. M. Sc.* 196: 211. 1938.

In an atypical case of diabetes the patient was restored from coma by 150 P. units of insulin together with adjunctive measures. Subsequently his tolerance remained remarkably high. Within one 24-hour period he received 3250 units, apparently the largest amount ever reported. A search was made for pituitary diabetogenic hormone by injections of extracts of the patient's blood and urine into depancreatized rats but these were without effect on animals' glycosuria.—R. G. H.

PARATHYROID

MACBRYDE, C. M. Treatment of hypoparathyroidism with dihydrotachysterol. *South. M. J.* 31: 724. 1938.

Though cases of tetany are relatively rare, many patients suffer from hypocalcemia, with its associated disabilities. Such cases present serious problems in treatment, for neither viosterol nor parathormone are satisfactory over long periods. The author reports on 4 patients successfully treated for at least 6 mo. with a derivitive of irradiated ergosterol. Hypocalcemia tetany can be successfully treated with small doses of dihydrotachysterol taken orally. The symptoms can be entirely relieved and the blood calcium can be maintained at normal levels. Larger doses are required if no calcium is given in addition to that normally contained in the diet. The methods of choice for the treatment of acute tetany are the intravenous injection of calcium salts and the intramuscular injection of parathyroid extracts. For the treatment of chronic tetany dihydrotachysterol has many advantages over parathyroid extract.—J. C. D.

MACBRYDE, C. M. Treatment of parathyroid tetany with dihydrotachysterol. *J.A.M.A.* 111: 304. 1938.

Seven female patients with chronic hypocalcemia and the symptoms of tetany were treated with dihydrotachysterol. Five had been under observation and treatment for some years. Their ages ranged from 21 to 56. In 6 of the patients the tetany occurred following thyroid operations, while in the youngest patient it was of the so-called idiopathic type. The patients were completely relieved. The previous duration of the symptoms of tetany ranged from 3½ to 17 years, and all previous therapy had been comparatively ineffective except for the temporary relief afforded by injections of parathyroid extract and Ca. Three patients had developed resistance to the parathyroid extract. Bilateral cataracts had developed in 3 of these patients previous to the dihydrotachysterol therapy. Two patients had suffered frequent severe epileptiform convulsions. The convulsions did not recur after treatment with dihydrotachysterol. Approximately normal serum Ca levels are now being maintained in the 7 patients with doses of from ½ to 1 cc. of dihydrotachysterol daily, supplemented daily by from 4 to 10 g. of calcium lactate or gluconate. At the present time, one patient has been free from symptoms for more than one year, one for 7 mo., 2 for more than 6 mo. and 2 for more than 3 mo. No tolerance to the drug has been noted. In several cases smaller doses than those originally used seem sufficient to keep the blood calcium normal. In no case was it necessary to increase the dose.—Abstract service *J.A.M.A.*

PICKHARDT, O. C., AND A. BERNHARDT. Treatment of postoperative tetany with dihydrotachysterol. *Ann. Surg.* 108: 362. 1938.

The authors report their experience with a 0.5% sesame oil solution of dihydrotachysterol in the treatment of 5 cases of tetany following surgery on the thyroid. The conclusion is that dihydrotachysterol has great value in the control of postoperative tetany. The symptoms of tetany can be alleviated within 48 to 72 hr. after treatment has been instituted. Therapy with dihydrotachysterol must be strictly individualized, i.e. the blood Ca level must be carefully checked at intervals before and after medication has been instituted. Until the maintenance dosage has been determined, the amount of dihydrotachysterol given by mouth may vary from 1 to 3 cc. every other day to 1 to 2 cc. weekly, depending on the severity of the deficiency. The authors have on occasion administered dosages as high as 10 cc. daily for a period of several days, but during this period the patient's serum Ca was checked carefully. The product is not harmless, and excessive dosage may well induce symptoms of hypercalcemia. A plea is made for the routine determination of serum Ca both preoperatively and postoperatively in all thyroidectomies. The authors found this new substance of definite value from both a subjective and an objective standpoint in the control of tetany. These observations, taken in conjunction with those of Holtz and his co-workers, would suggest that dihydrotachysterol merits further chemical study and it is recommended for the treatment of postoperative hypoparathyroid tetany.—Abstract from *J.A.M.A.*

ROJAS, P., AND J. F. MANFREDI. Parathyroid grafts in experimental hypoparathyroidism (La greffe parathyroïdienne dans l'hypoparathyroïdisme expérimental). *Compt. rend. Soc. de Biol.* 127: 702. 1938.

Successful parathyroid grafts were made in the infrahyoid muscles or beneath the capsule of the kidney in dogs. Presumably the dogs were parathyroidectomized, as one of the tests of a successful graft was the occurrence of tetany after removal of the transplant. It is not stated whether the grafts were autoplastic or homoplastic. Grafts were studied histologically at different periods ranging from a few hours to 15 mo., and blood Ca and K were also determined after removal of the grafts.—E. Cutuly.

THYROID

GJESSING, R. Disturbance of somatic functions in catatonia with a periodic course, and their compensation. *J. Ment. Sc.* 84: 608. 1938.

Over a period of 13 yr. the author has carried out metabolic studies on a group of psychotic patients. Among these were 24 subjects of periodic catatonia. The acute episodes were found to be characterized by nitrogen retention. With a view to preventing this retention the patients were treated with thyroxin or desiccated thyroid to hold the nitrogen in low equilibrium. This was usually accomplished with a dosage that kept the oxygen consumption rate at 10 to 15% above calculated normal. The dosage of thyroxin was 2 mg. intravenously on the first day with gradual increase to 40 to 50 mg. Having got the patient adjusted with thyroxin he could be held in adjustment with thyroid substance. The author concludes that, while thyroid medication is useless in most forms of schizophrenia, in this type of catatonia the patients are rendered and kept symptom-free.—R. G. H.

KUMMER, H. Early morphological changes in the thyroid following a single injection of thyrotropic hormone (Die morphologischen Frühveränderungen an der Schilddrüse nach einer einmaligen Injektion von thyreotropen Hormon). *Endokrinol.* 20: 326. 1938.

Thyrotropic hormone of the hypophysis was given to guinea pigs by intraperitoneal injection; the thyroid glands were later removed and studied histologically. It was found that a single injection of 20 guinea pig units or 2 mg. of thyrotropic hormone into a sexually immature male guinea pig calls forth in 2 hr. a slight, and in 4 hr., a definite increase in the height of the follicular epithelium.—W. J. A.

LEWIS, R. A. The effect of hyperthyroidism upon the metabolism of vitamin C. *Bull. Johns Hopkins Hosp.* 63: 31, 1938.

The vitamin C excretion of 5 hyperthyroid patients on a constant diet was less than that observed in normal individuals. Following subtotal thyroidectomy the excretion of vitamin C rose, reaching normal values in 4 of the 5 patients, after several weeks.—A. G.

MEMBRIVES, J. R. Thyroid and gonadotropic power of the rat pituitary (Thyroïde et pouvoir gonadotropique de l'hypophyse du rat). *Compt. rend. Soc. de biol.* 127: 695. 1938.

The gonadotropic potency of the pituitaries of a series of rats weighing 140 to 220 gm. was investigated by implantation of the glands into immature females. The ability of the hypophyses to cause ovarian development and consequent vaginal canalization was found to be decreased by thyroidectomy. increased by ingested thyroid substance, decreased by folliculin, and increased by castration.—E. Cutuly,

PUPPEL, I. D., AND G. M. CURTIS. The iodine balance in nodular goitre. *J. Clin. Invest.* 17: 729. 1938.

Iodine balance studies were done on low iodine intake, in 2 non-toxic nodular goiter, 2 toxic nodular goiter, 3 exophthalmic goiter, and 1 hypothyroid patients. Findings in 3 normals have previously been reported. All are in negative iodine balance under these conditions, but the non-toxic cases resembled the normal individuals. The differences between the nodular and exophthalmic types were that the nodular types showed a greater iodine excretion in proportion to the elevation in basal metabolism, and that the exophthalmic types showed a greater increase in fecal iodine loss than the nodular cases. Following thyroidectomy the iodine metabolism returns rapidly toward normal. The iodine excretion in hypothyroidism, when therapy is just withdrawn, resembles that of normals and those with toxic nodular goiter.—E. L. S.

REMINGTON, R. E., AND J. W. The effect of enhanced iodine intake on growth and on the thyroid glands of normal and goitrous rats. *J. Nutrition* 15: 539. 1938.

Young rats produced on a normal colony diet containing 265 parts per billion of I were fed added amounts of I as NaI and as dried haddock, up to 40 times the amount present in the colony diet. No significant changes in fresh weight, dry matter or I content of the thyroid were observed, and it is concluded that previously published normal values may be relied upon. The enhanced I intake was without effect on growth or the utilization of food for growth. Other groups of rats reared on an I deficient régime, and goitrous, were fed either the colony diet with enhanced I, or the goitrogenic diet with enhanced I content. In all cases the constants on the thyroid tended to return toward normal, but either the recovery was not complete as to size and dry matter content, or there was an excessive storage of I in the glands. The normal relationship between weight and I content of the thyroid does not hold for 'cured' animals.—Author's Abstract.

ROSS, R. Relation of the thyroid to reproduction (Die Beziehungen der Schilddrüse zur Fortpflanzung). *Arch. f. Entwcklngsmechn. d. Organ.* 137: 771. 1938.

Castration of immature guinea pigs increased the amount of the colloid in the thyroid gland. Castration in rats produced only slight atrophy of the thyroid, while thyroidectomy resulted in no significant histological change in the reproductive organs but marked loss of sexual interest. Chronic thyroidectomy

in immature male rats was found associated with general body retardation. In female rats thyroidectomy decreased the number of corpora lutea and size of the uterus, lengthened the diestrous interval and retarded growth and development in the immature rat. Removal of ¾ of the thyroid gland proved compatible with normal littering in ⅓ of the animals.—A. T. R.

SCHNEIDER, E. Vitamin B and experimental hyperthyroidism (Vitamin B_1 und experimentelle Hyperthyreose). *Klin. Wchnschr.* 17: 542. 1938.

Because of his inability to confirm Sure and his coworkers on the beneficial effect of B in experimental hyperthyroidism, the author studied the effect of B deficiency upon the thyroid histology and liver glycogen in the rat. After 5 to 7 weeks on the B-deficient diet, the rat thyroid was found in the 'resting stage' and the liver glycogen was normal. This was at a time when the symptoms were pronounced. Such glands responded normally to the injection of anterior pituitary extracts. Terminally, there was a drop in liver glycogen.—M. H. F.

SCIAKY, I. Experimental hyperthyroidism in different animals (Hyperthyroïdisme expérimental chez différentes espèces animales). *Ann. d'anat. path.* 15: 165. 1938.

A description of the pathological findings in 6 guinea pigs, 4 rabbits, 3 cats and 5 rats, in some of which acute intoxication was produced by large doses of thyroxine and in others chronic intoxication by small doses of thyroxine. All lost weight (6 to 47%). In most cases recognizable changes occurred in the liver and heart. The islands in the pancreas of the guinea pig reacted differently to large and small doses, undergoing necrosis in the former and hyperplasia in the latter.—A. T. R.

STÉVENIN, H., AND B. GINSBOURG. Blood glutathione in thyroid disorders (Le glutathion sanguin dans les affections thyroïdiennes). *Compt. rend. Soc. de biol.* 127: 382. 1938.

From results on 5 human subjects with thyroid adenomas and on 5 hyperthyroid individuals it was concluded that disappearance of oxidized glutathione from the blood is not a specific indication of hyperthyroidism.—E. Cutuly.

WILTRAKIS, G. A., AND A. V. PARTIPILO. Non-myxedematous hypothyroidism. Case reported with psychosis. *Illinois M. J.* 74: 359. 1938.

The case described occurred in an emaciated unmarried male schizophrenic of 23 with a B.M.R. of −45%. Under thyroid medication the metabolic rate returned to normal, there was a gain of 67 lb. in 3 mo. and an improved mentality. During the past 2 yr. thyroid medication has been continued, with maintenance of the results achieved.—I. B.

WISE, B., AND H. E. HOFF. The effect of the autonomic hormones on the thyrotoxic heart. *J. Pharmacol. & Exper. Therap.* 64: 217. 1938.

Electrocardiograms were taken of 9 normal rabbits before and after the administration of Mecholyl. The resting heart rate, averaging 260 per min., was increased to 330 in some cases, 15 to 30 sec. after the subcutaneous administration of 5 to 10 mg. of Mecholyl. Between 1 and 2 min. after injection, the heart rate was diminished followed by acceleration. The heart rate returned to normal in 20 to 30 min. After feeding 0.3 g. of desiccated thyroid every other day for 19 days, injection of Mecholyl produced immediate acceleration which was followed by extreme slowing (as low as 93 per min.) and sinus arrhythmia. Brief periods of acceleration were sometimes present during the sinus arrhythmia. Epinephrine, 1 cc. of 1:10,000 intravenously in a group of 10 normal rabbits, caused first an enormous slowing (heart rate, 30 per min. in one instance) lasting for not longer than 15 sec. and then a rapid rate varying between 300 to 400 per min. with occasional extra systoles or coupling. In rabbits, which were thyrotoxic due to the repeated feeding of thyroid, epinephrine caused a slight transient slowing of the heart followed by tachycardia, the heart rate being 400 to 500 per min. Ventricular fibrillation was produced in 2 of these 10 animals. The exaggerated response of the thyrotoxic heart to Mecholyl and epinephrine may be due to the synergistic effect of the existing acetyl-choline and sympathin or the increased sensitivity of the thyrotoxic heart.—E. L.